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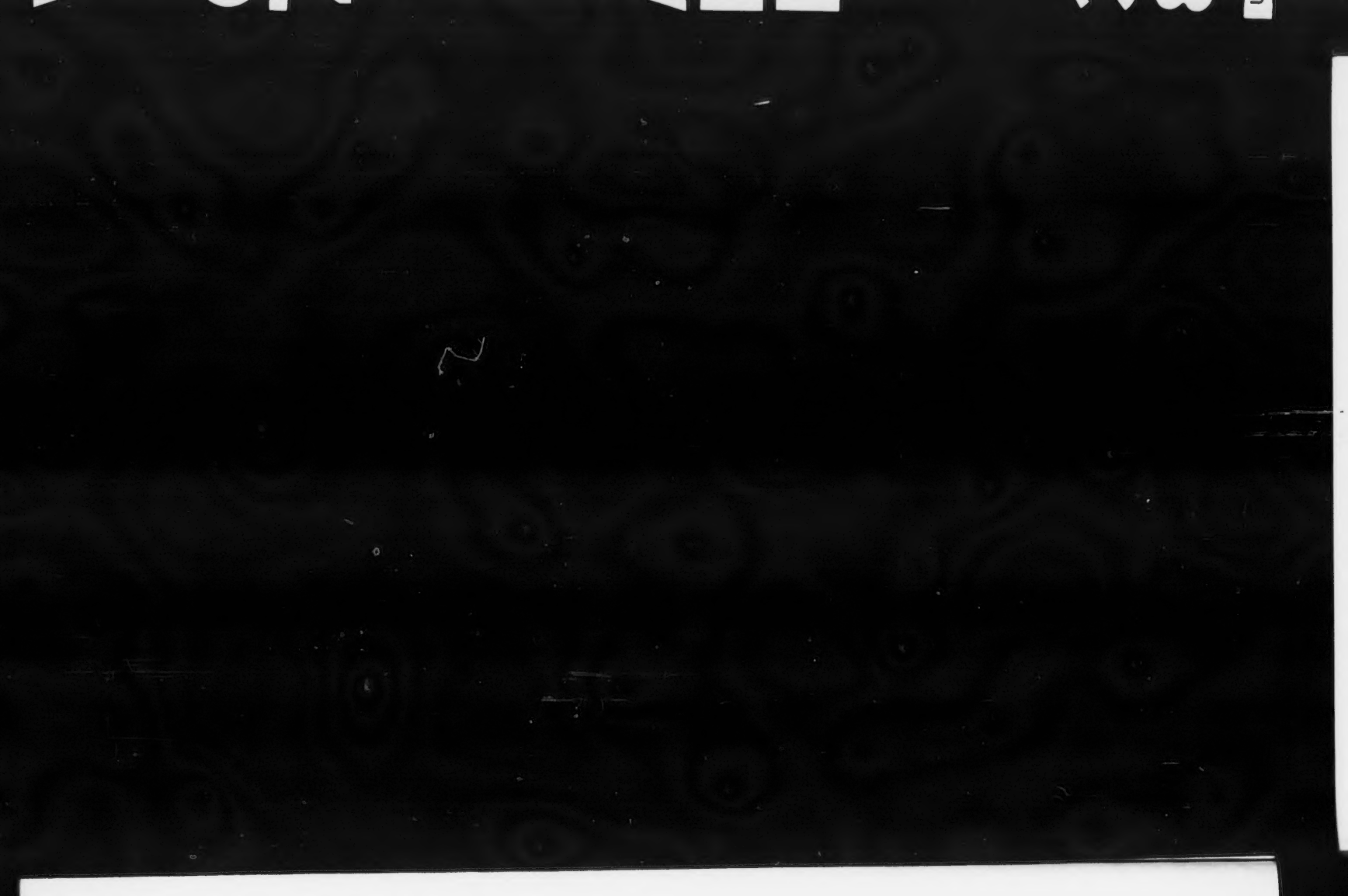

Canadian Journal of Research

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VOL. 16, SEC. A. APRIL, 1938 NUMBER 4

APPLICATION OF QUARTZ CRYSTALS TO THE MODULATION OF LIGHT[1]

BY D. W. R. MCKINLEY[2]

Abstract

The resonating *X*-cut quartz crystal has been used as a self-compensator for white light modulation.

The properties of the 49° cut quartz crystal are described. This crystal combines low power input and stable high frequency with a wide light aperture and improved light output. For many purposes a convergent beam of light may be used, without the need of a compensating device.

Vibrating quartz crystals have the property of dynamically rotating the plane of polarization of a plane polarized beam of light passing along the optic axis. The light emerging from a polarizer-quartz, plate-analyzer system is transmitted in flashes of twice the crystal frequency.

1. The X-cut Quartz Crystal Light Modulator

A plane parallel beam of white light is polarized by the polaroid P_1 (or by a Nicol prism) and passes through the quartz crystal C, making a small angle of 2° or 3° with the Z (optic) axis, Fig. 1. It is reflected from the mirror M_1 and passes back through the crystal to the mirror M_2, which reflects the light

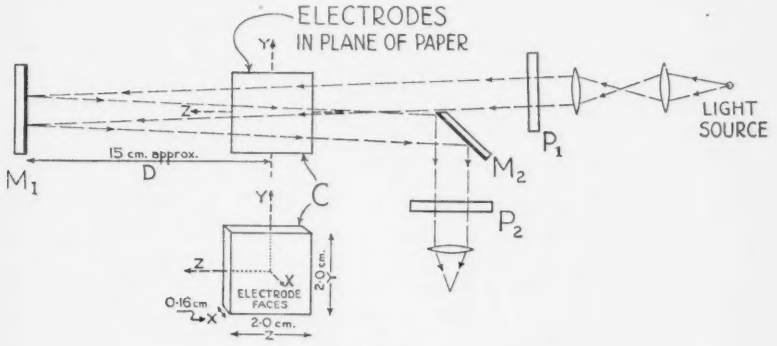

FIG. 1.

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to one side. The light is then passed through the analyzing polaroid P_2, and may be focused as desired. The polaroids are so arranged that the light cut-off is complete when the crystal is not oscillating, provided that the beam is parallel.

When the output from a vacuum tube oscillator is applied to the crystal at its fundamental resonant frequency, light appears uniformly over that part of the crystal seen through P_2. As the oscillator voltage is increased, the intensity of the transmitted light increases. The optimum effect occurs with inputs of the order of 20 to 25 watts.

The principle of this quartz cell is not the same as that of the Kerr cell, in which the observed light depends not only on the applied voltage but also on the distance D, reaching a maximum when D is one-eighth of the electrical wave-length of the alternating voltage. The intensity of light transmitted through quartz does not vary in the same manner with the distance D, by virtue of the complex rotation pattern of the vibrating crystal. Rotation pattern is an expression used to describe the appearance of the vibrating crystal when placed between polaroids and viewed directly in monochromatic light. The bright spots represent the vibration loops of the standing waves in the crystal.

Light that passes through the more active portions of the quartz on its first transmission will, in general, pass through parts of lesser activity on its return, and is consequently no longer polarized perpendicular to the polarization plane of P_2. On the other hand, light going through the least active portions of the crystal on its initial passage will be returned through a more active part, and therefore also suffers a net change of polarization. So the whole observed crystal surface appears strongly illuminated, and all trace of pattern disappears.

The usual arrangement up to the present has been to pass the light once through the active quartz plate and then through a quartz slab of opposite rotatory power; this annuls the static light rotation of the first plate and permits white light cut-off. Because the above-mentioned reflection method utilizes every portion of the crystal, the light efficiency is greater than it is in the latter method where the light passes but once through the quartz and compensator, because in this arrangement only the active portions of the excited crystal transmit light.

The upper limit of useful frequencies seems to be about 2.5 megacycles, since beyond this the crystal becomes too thin to admit the beam of light. If the frequency in kilocycles per second is f and the thickness in centimetres is d, then $f = \frac{287}{d}$.

It is possible to modulate the oscillator voltage at audio frequencies and therefore to modulate the light beam at these frequencies, when the high frequency light interruptions are used as a carrier. The high value of Q of the quartz resonator $\left(Q = \frac{\omega L}{R}\right)$ may be reduced considerably by tightly clamping the crystal and thus increasing the sideband range. In a test, the

driving frequency could be varied 5 kc. from the mean of 2000 kc. without appreciably affecting the light output. This frequency range would be useful for speech frequencies. However, it is unlikely that this quartz light cell would compare favorably with the usual moving metal ribbon, glow lamp, and other types of light cells for audio frequencies. The quartz cell will respond to a small band of very high frequencies.

A suggested application is its use as a high frequency chronograph to put a time base on high speed film. It has been used in this laboratory in conjunction with a tuned photocell in measurements on the velocity of light, though the crystal described in the second part of this paper has been more satisfactory for the purpose.

2. The 49° Cut Quartz Crystal Light Modulator

To obtain the most pronounced electro-optical effect, the light should pass along or close to the optic axis. This condition is satisfied in the X-cut type. However, to secure higher fundamental frequencies it is necessary to reduce the crystal thickness; this unavoidably narrows the light aperture.

A thick crystal may be driven at higher harmonics to obtain higher frequencies of interrupted light, but there are three disadvantages to this procedure. First, the power required to excite the crystal harmonically is much greater than that needed to excite the fundamental frequency, if the same resultant light intensity is assumed. Second, the line grating formed in the crystal by the nodes and loops of harmonic vibrations causes much of the light to be deviated from the main beam, and for some applications this is undesirable. Third, increased power input results in increased heating of the crystal, and, owing to the large temperature coefficient of frequency for the X-cut type (20 to 35 cycles per megacycle per °C.), the frequency will vary rapidly unless elaborate precautions are taken to maintain a constant temperature.

The light aperture may be made independent of the fundamental frequency by so orientating the cut that the Z-axis is inclined at an angle to the faces of the plate. However, as the angle of inclination is increased the piezoelectric effect decreases and becomes zero when the Z-axis is perpendicular to the faces. The angles should be at least 49° in order that light entering at a small angle to the faces will be refracted through the crystal in the same, or nearly the same, direction as the Z-axis.

Lack and co-workers (1) showed that the unwanted coupling between the x_y and z_x strains is zero at $+31°$ and $-60°$; consequently, there is less danger of fracturing crystals when the orientations have these values. These writers have also calculated the temperature coefficient of frequency in terms of the inclination of the Z-axis, and have shown that at $+35°$ and $-49°$ the desired shear vibration has zero temperature coefficient. Of these, the 49° cut is useful for optical purposes, and the angle is also sufficiently close to that of the 60° cut to minimize undesired couplings and permit greater power inputs than does either the X or Y cuts.

The 49° cuts used in this work have an average thickness of about 0.063 cm. and are 2.54 cm. square, with frequencies between 3725 and 4050 kc. The crystal is mounted between two brass blocks, B_1 and B_2 (of dimensions 3.2 by 3.2 by 0.67 cm.), which serve as electrodes. A hole, 1.3 cm. in diameter, is drilled in the centre of each electrode. The blocks are channelled, Fig. 2, to enable light to enter at a small angle to the crystal faces. The crystal is properly orientated between the electrodes, the two plates are clamped together in an insulated holder, and pressure is applied by means of two set screws. The amount of clamping is not usually critical.

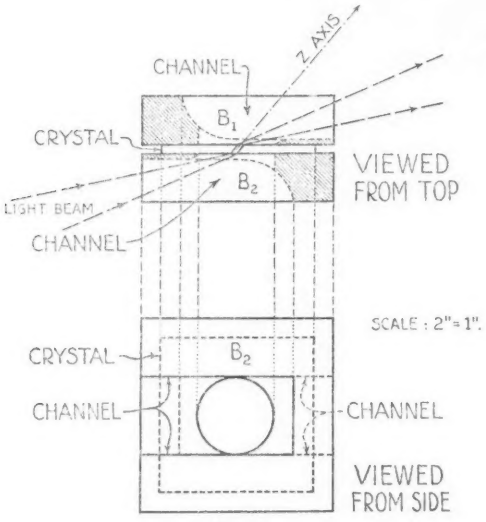

FIG. 2.

Owing to the thinness of the crystal, it is no longer necessary to pass light through in a parallel beam. Light may be focused through it, provided that the angle of the light cone is not too large.

The effective maximum thickness of the optical path is about 0.082 cm., and in general is less because the light does not enter at grazing incidence. This thickness corresponds to a rotatory dispersion of the visible spectrum of eight to ten degrees. This means that in practice good cut-off of white light may be obtained for many purposes without the use of either compensator plate or the reflection method described in the first part of this paper.

In fact, for operation with a tuned photocell, best results are obtained without any compensating medium between the polaroids; this is probably due to reflection and absorption losses of the compensator. However, if absolute panchromatic cut-off is desired, a solution of a sugar of the opposite rotatory power is quite effective with crystals of this thickness.

Very little exciting power is necessary for these crystals. Two to five watts of radio frequency input will drive them to strong light output. Owing to their design, the crystals have withstood, without fracture, voltages as high as the flashover voltage between the electrodes. Normally they are operated far below this breakdown voltage, since increasing the input beyond about ten watts does not proportionately increase the output.

As the power consumption is small, the crystals do not heat excessively and require no cooling system, even for several hours of operation. This fact, together with the zero temperature coefficient, ensures a very stable oscillation frequency. Tight coupling is used between the driving oscillator and the crystal circuit; consequently, the oscillator locks with the crystal frequency.

For the past year these 49° cut crystals have been used at the McLennan Laboratory in a method for measuring the velocity of light, and have given satisfactory results. Measurements are as yet incomplete, but at present the indicated accuracy of the method is of the order of that obtained in the Kerr cell and rotating mirror experiments. Many more observations will be made before an account of this work is published.

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THE INFLUENCE OF THICKNESS ON THE MEASURED THERMAL CONDUCTIVITY OF FIBREBOARD AND ROCK WOOL[1]

By J. D. Babbitt[2]

Abstract

The thermal conductivity of samples of rock wool and fibreboard of various thicknesses (0.5 to 2.0 in.) was measured by means of a hot-plate apparatus. It was found that when surface effects were eliminated the conductivity obeyed Fourier's law.

It has been found recently, (1, 2, 3) that there is a considerable change in the measured thermal conductivity of a material when the test is made on samples of different thicknesses. Corkboard, fibreboard, and rock wool were tested, and although practically no change was found in the value of k for corkboard as the thickness was increased from $1\frac{1}{2}$ to 4 in., yet the results indicated that "the conductivity of a 2-inch fibreboard is approximately 23% greater than that of a $\frac{1}{2}$-inch board of the same material. In matted materials, consisting of grasses or other fibres stretched between layers of papers, the difference is about 50%". These figures are so large that they are of importance not only for purposes of practical insulation but also for theoretical considerations.

It has always been implicitly assumed that Fourier's law,

$$Q = kA \frac{dt}{dx}, \tag{1}$$

holds for the transmission of heat through solid materials, and that the quantity of heat transmitted is inversely proportional to the thickness of the material. In Equation (1), Q is the quantity of heat transmitted, k is the coefficient of conductivity, A is the area, and $\frac{dt}{dx}$ is the temperature gradient. It has been assumed that this law holds for the flow of heat through fibreboards, where the insulating property is due to the entrapped air cells, and it is even applied to fibrous materials such as rock wool, where the air cells constitute a much greater proportion of the volume. It was supposed that as long as the dimensions of the air cells were small in comparison with the thickness of the material, the thermal resistance of the various cells would be additive; thus the total resistance would be proportional to the thickness. When the density of a material such as rock wool becomes so low that convection currents can flow through the sample as a whole and are not confined to the individual air spaces, then the conductance would be expected to approach that of an unfilled air space and to vary with the thickness in a similar manner. In practice, however, density

[1] *Manuscript received February 13, 1938.*
Contribution from the Division of Physics and Electrical Engineering, National Research Laboratories, Ottawa, Canada.

[2] *Physicist, National Research Laboratories, Ottawa.*

of packing of the mineral wools used is great enough to prevent the flow of such convection currents through the material, and therefore the conductance should vary inversely as the thickness, according to Fourier's law.

The suggestion has been made that the effect in fibreboards may be due to the decreasing effect of surface resistance as the thickness becomes greater. There are two effects that should be distinguished.

(*a*) In the first place, it is known that the conductivity of fibreboards is decreased if the fibres can be made to lie paraliel to the surface, so that the heat has to flow across the fibres and from fibre to fibre instead of along the individual fibres. This result has been demonstrated experimentally by Finck (4). He showed that the conductivity of some materials can be more than doubled by arranging the fibres parallel to the direction of the heat flow. Now, owing to the method of manufacture of fibreboards, the fibres in those layers near the surface tend to lie parallel to the surface, while those in the body of the material are directed at random. The surface layers would thus have a greater thermal resistance than the material in the centre of the sample, and this would cause thin samples to show a conductivity lower than that of thick ones.

(*b*) The second surface effect that would change the measured value of the conductivity is purely experimental and is inherent in the method of testing. The standard method of measuring the thermal conductivity of homogeneous building materials is by means of a hot-plate apparatus, in which the quantity of heat flowing across a slab of the material placed between a hot and a cold plate maintained at definite temperatures is measured. In such an apparatus it is impossible to maintain perfect thermal contact between the plates and the samples, and this slight temperature drop is neglected. In effect, it is assumed that the slight difference between the conductance of the film of air separating the samples and the plates and that of a film of the material of the same thickness is negligible (6). This assumption is quite true for all samples having small conductance, but with poor insulators or with thin samples this surface effect becomes relatively greater and may become large enough to influence the value obtained for the conductivity. If this should occur it would cause the measured conductivity of thin samples to appear less than that of thicker samples, since the conductivity of air is less than that of the material.

When these two effects have been eliminated from the measurements on the conductivity of samples of a homogeneous material, the measured conductivity should be independent of thickness (Fourier's law). Since changes of 23 and 50% in the conductivity seemed to be larger than one would expect from either of the above-mentioned effects, and, since if they are to be found, they are much too large to be neglected, it seemed to be well worth while to investigate the problem further.

Method of Measurement

Since in all insulating materials such as fibreboards and corkboards there is a substantial variation in the conductivity of individual samples owing to

small local variations in material, density, arrangement of fibres, and other factors, it is important in testing for the effect of thickness to eliminate these variations if possible. The plan was therefore adopted of beginning a series of measurements with a thick sample and reducing the thickness for each subsequent measurement. This reduction in thickness of the fibreboards tested was effected by means of the sanding apparatus of the International Fibre Board Limited, Ottawa. Through any one series of tests, therefore, the measurements were made on the same identical sample.* The thickness of the sample of the rock wool in bat form was reduced by pulling off the outer layers of the wool until the sample was of requisite thickness; in each test the density of packing of the wool was the same. No provision was made to obtain an identical sample of granulated wool for each test, but great care was taken that the density remained the same, and that the wool was packed as nearly as possible in a similar manner.

The samples of fibreboards were always in a bone dry condition at the beginning of the test, and it was found that the increase in moisture during the test was very small. It was not considered necessary to dry the rock wool for the tests, since the absorption of moisture by this material is negligible.

The tests were all made in the hot-plate apparatus of the National Research Laboratories which has been described in detail elsewhere (5). It consists of a hot-plate, 1 ft. square, surrounded by a guard ring 3 in. wide. The dimensions of the face of the sample must therefore be 18 by 18 in. The rock wool was placed in wooden frames constructed of strips of wood $\frac{1}{2}$ in. thick. The width of these strips varied according to the desired thickness of the sample. The two faces of the frames were covered with brown kraft paper 0.005 in. thick. The thickness of all samples was determined by measuring the distances between the outside surfaces of the plates, with and without the samples in place. The difference in the two measurements was taken as the thickness of the sample. Measurements were made at each of the four corners of the plates, and the mean value was taken as the thickness. The pressure on all samples was the same, being that of a large weight attached to the plates by means of pulleys.

Results

The results obtained in these measurements are tabulated in Table I. Two samples of fibreboard were tested, both being supplied by the International Fibre Board Limited. Fibreboard I was an old sample that had been in the laboratory for more than two years. Fibreboard II was manufactured recently, and was taken from a commercial batch. The two surfaces of this sample had already been smoothed by sanding when originally supplied. It is to be noticed that there is an increase in the conductivity of Fibreboard I when the thickness is reduced from 2 to 1.5 in. The changes in the conductivity as the thickness is reduced from 1.495 to 0.276 in. are about what

* *There would be, of course, small local variations in the nature of the material in different parts of the fibreboards; it is impossible to eliminate these variations.*

one might expect from observational errors and local changes in the sample.* The conductivity of Fibreboard II remained practically constant in all tests. The behavior of these fibreboards can be quite easily explained. There was evidently in Fibreboard I a surface layer in which the fibres were arranged

TABLE I

Mean temp., °F.	Temp. diff., °F.	Thickness, in.	k, B.T.U. per hr. per sq. ft. per °F. per in.	C, B.T.U., per hr. per sq. ft. per °F.	$R = \frac{1}{C}$
Fibreboard I: density, 17.8 lb. per cu ft.					
59.1	61.7	2.081	0.409	0.197	5.09
58.7	62.4	1.495	0.429	0.287	3.48
55.0	54.0	0.995	0.436	0.438	2.28
56.7	58.5	0.759	0.432	0.569	1.76
54.7	54.4	0.486	0.424	0.870	1.15
53.6	52.3	0.276	0.416	1.506	0.66
Fibreboard II: density, 12.2 lb. per cu ft.					
56.8	58.6	1.970	0.357	0.181	5.53
56.6	58.1	1.512	0.359	0.237	4.21
56.5	58.1	1.018	0.350	0.344	2.91
55.4	55.9	0.751	0.355	0.473	2.11
56.6	58.2	0.495	0.344	0.694	1.44
Rock wool in bat form: density, 7.1 lb. per cu ft.					
59.0	58.1	2.012	0.225	0.112	8.98
56.2	57.5	2.002	0.228	0.114	8.83
56.7	58.3	1.527	0.236	0.155	6.47
58.5	62.0	1.073	0.235	0.219	4.57
56.1	57.3	0.517	0.233	0.450	2.22
55.6	56.3	0.276	0.231	0.838	1.19
Granulated rock wool: density, 7.9 lb. per cu. ft.					
55.0	55.0	2.005	0.283	0.141	7.08
56.9	58.8	2.007	0.276	0.138	7.27
54.2	53.5	1.535	0.266	0.173	5.77
53.6	52.2	1.004	0.271	0.270	3.71
53.7	52.5	0.523	0.266	0.509	1.96

parallel to the surface. This was removed in the first sanding, with the result that the conductivity increased. The 0.5 in. of fibreboard that was removed to reduce the thickness was taken off one surface of the sample. In the second reduction of thickness the material was removed from the opposite surface, and there is another small increase of conductivity that may be due to the effect of fibre arrangement, but is not so large as the first reduction and may possibly be fortuitous. This increase in conductivity with decreasing thick-

* *Too much reliance must not be placed on the results obtained with the* $\frac{1}{4}$ *in. sample, since this is a rather thin fibreboard and the conductance is large.*

ness is just opposite to the effect that Allcut reports, but it is probably a similar effect. Evidently Allcut tested samples in all of which the fibres in the surface layers were parallel to the surface; consequently, in the thinner samples the conductivity is smaller owing to increased influence of the surface layers. In our work the surface layer was removed to reduce the thickness; this gave an

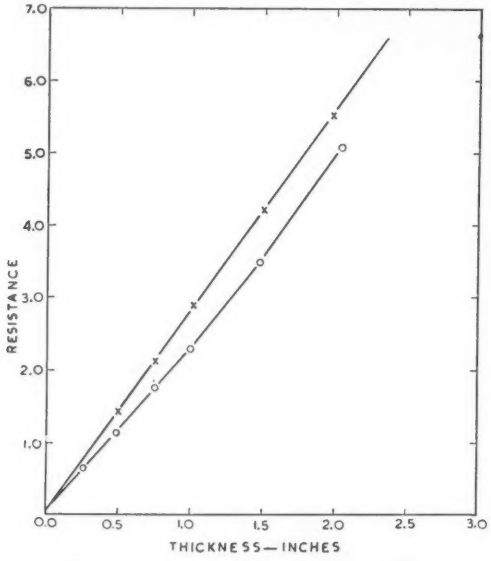

FIG 1. o, *Fibreboard I;* x, *Fibreboard II.*

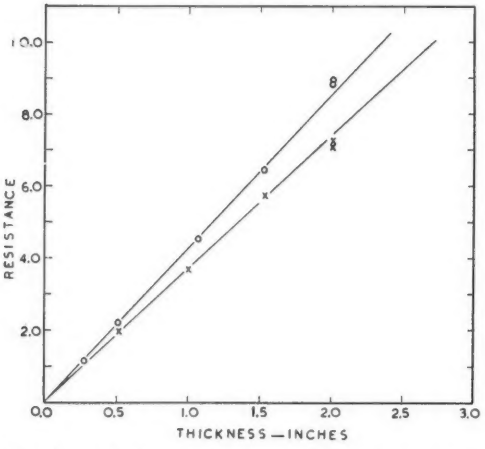

FIG. 2. o, *Rock wool in bat form;* x, *rock wool granulated.*

increased conductivity. Fibreboard II shows no change in the conductivity with thickness, presumably because the surface layers were removed in the preliminary sanding during the manufacture of the sample. The two samples of rock wool also show no appreciable change in conductivity.

In Figs. 1 and 2 these results have been presented in a different manner. Here the resistance R of the samples has been plotted against the thickness. If the thermal conductivity of the material accurately obeyed Fourier's law, the points should give a straight line; any curvature denotes a departure from this law. The effect of the surface layers in Fibreboard I is plainly evident. This method of representing the results is also instructive in that it gives a method of determining the effect of the temperature drop from plate to sample, as explained above. If there were no errors due to this, the curve would pass through the origin; this would show that with no material between the plates the heat transfer would be infinite. When the surface effect is present, some resistance, even with no material between the plates, should be found; in other words, the curve would not pass through the origin but would cut the y-axis at a finite point. In our samples this effect, as shown above, is very small; in fact, in the rock wool it is too small to be distinguished. There is some evidence of such an effect in the fibreboards, but it is so small that it comes within the limits of the observational error. These results are extremely satisfactory in that they prove conclusively that we are justified in neglecting the resistance between sample and plate in our hot-plate tests, at least with fibreboards and rock wool. When this end effect is not negligible, the true conductivity of the material can be accurately determined from this type of graph by using the slope of the curve. This, of course, could be done only when the material obeys Fourier's law, and thus gives a linear relation between resistance and thickness.

These experiments show that when surface effects have been eliminated the transmission of heat through fibreboards and rock wool (of sufficient density) may be treated as a true conduction obeying Fourier's law. Thus, in the calculation of the resistance of a wall in which these materials are incorporated, the use of a formula that treats the resistance as the thickness divided by the conductivity is quite legitimate.

Acknowledgment

The author wishes to thank Mr. E. H. J. Barber of the International Fibre Board Limited, who kindly superintended the sanding of the fibreboard samples, for his co-operation.

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INDUCED ASYMMETRY AND OPTICAL RESOLUTION OF 2-PHENYLPYRIDINE DERIVATIVES[1]

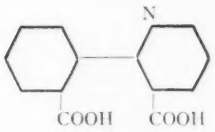
BY J. G. BRECKENRIDGE[2] AND O. C. SMITH[3]

Abstract

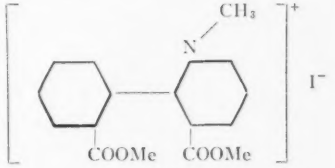
It was shown in 1927 that quinine, when combined with a diphenic acid derivative, gave a salt with an optical rotation in the opposite direction to that of the free alkaloid ("Kuhn's effect"). The present authors have prepared the salts of four of the cinchona alkaloids with 2-phenylpyridine-2′:3-dicarboxylic acid, and find similar results. In addition, a partial optical resolution of a derivative of this acid has been effected, the dextro-form of the methiodide of the dimethyl ester having been isolated.

The first example of the type of so-called asymmetric induction described here was observed by Kuhn and Albrecht (4), when, on preparing the quinine salt of 4:4′-dinitrodiphenic acid, they obtained a dextrorotatory product having $[\alpha]_D^{22^\circ} + 110^\circ$ in chloroform. The theory was advanced that the alkaloid induced an asymmetric configuration in the remainder of the molecule, the asymmetry disappearing on removal of the alkaloid, since an inactive acid only could be liberated from the salt. Since then other examples of what is known as "Kuhn's effect" have been observed—Bell and Robinson (1) with 4-nitrodiphenic acid, and Lesslie and Turner (5) with diphenic acid itself—the free acid in each case not having the necessary substituent groups in the 6-positions to show the well known asymmetry of various other derivatives. This subject has been reviewed by Ritchie (7).

A compound of a type similar to diphenic acid is 2-phenylpyridine-2′:3-dicarboxylic acid (I).



(I)



(II)

It was thought that it might be of interest to form the salts of (I) with several of the cinchona alkaloids, and possibly provide another example of Kuhn's

[1] *Manuscript received February 12, 1938.*
Contribution from the Department of Chemical Engineering, University of Toronto, Toronto, Canada. Part of this paper is an abstract of a thesis submitted by O. C. Smith in partial fulfilment of the requirements for the degree of B.A.Sc.

[2] *Instructor, Department of Chemical Engineering, University of Toronto.*
[3] *Fourth year student, Department of Chemical Engineering, University of Toronto.*

effect. Accordingly, the salts of (I) with quinine, quinidine, cinchonine, and cinchonidine were prepared, examined in the polarimeter, and their specific rotations calculated. The results obtained with the free alkaloids and their salts are given in Table I.

TABLE I

SPECIFIC ROTATIONS OF THE FREE ALKALOIDS AND THEIR SALTS

		Quinine	Quinidine	Cinchonine	Cinchonidine
Alkaloids					
	c	2.14	2.10	0.37	2.12
	$[\alpha]^{22}_{5461}$	−143°	+258°	+277°	− 94°
Salts					
	c	3.03	2.90	3.04	2.96
	$[\alpha]^{22}_{5461}$	+244°	+206°	−250°	+303°
	$[\alpha]^{22}_{5780}$	+209°	−175°	−211°	+259°
	$[\alpha]^{22}_{4358}$	+476°	−412°	−516°	+617°
	$[M]^{22}_{5461}$	+2175°	−1335°	−2078°	+2519°

The rotations were measured in chloroform in a 20 cm. tube, and, with the exception of cinchonine, the concentrations used were such that the amount of alkaloid present, both free and in the salt, would be approximately the same.

Lesslie and Turner (5) observed mutarotation both in the initial mixture of diphenic acid and alkaloid, and in solutions of the crystallized salts. In the present work, solutions of the salts showed a constant rotation, the first reading being taken three minutes after the salt was wetted, a mixture of two volumes of ethanol to one volume of chloroform being used, but the mutarotation in the freshly prepared solution was just observable. Equivalent quantities of the acid and each alkaloid were dissolved in ethanol, mixed, made up to a known volume, and the first readings taken one and one-half minutes after mixing. Observations were made at intervals for 48 hr. The rotation increased noticeably during the first two minutes of observation, but thereafter remained constant. Apparently with (I) the activation process is very much more rapid than it is with diphenic acid, although the two compounds are very similar.

Attempts have been made to resolve derivatives of (I), use being made of the conveniently situated nitrogen atom. Chalmers, Lions, and Robson (2) worked with the strychnine and brucine salts of the methosulphate, but could obtain no evidence of resolution, and the present authors attempted the preparation of salts of the cinchona alkaloids with both the metho- and etho-sulphates, but were unsuccessful. A crystalline salt could not be isolated, the usual result being the recovery of the metho- or etho-sulphate of the alkaloid. However, a partial resolution of the methiodide of the dimethyl ester (II) was effected by the use of silver α-bromocamphor-π-sulphonate,

the dextro-form of (II) being isolated. The silver salt of (I) was treated with methyl iodide to obtain the ester, which was heated with more methyl iodide in a sealed tube, and the resulting methiodide was treated with silver bromocamphor sulphonate. The crystalline bromocamphor sulphonate obtained showed a rotation in chloroform ($c = 2.0$) of 4.28° for λ 5461Å, from which the specific rotation $[\alpha]^{22}_{5461} = +107°$, and $[M]^{22}_{5461} = +637°$. The methiodide, recovered by shaking a chloroform solution of the bromocamphor sulphonate with aqueous potassium iodide and precipitating with ether, gave a rotation in chloroform ($c = 0.30$) of 0.94° for λ 5461Å, from which $[\alpha]^{22}_{5461} = +156.7°$, and $[M]^{22}_{5461} = +648°$. All rotations were measured in a 20 cm. tube. The value recorded for the methiodide was unaltered after two recrystallizations, and an experiment carried out in ethanol solution showed no change in rotation after heating to 70° C. for three hours.

This is evidently another process of activation rather than resolution, similar to several already recorded (6), whereby one form only crystallizes (in this case the *d*-bromocamphor sulphonate *d*-complex). In previous instances it was found possible to obtain the other form by changing the solvent or method of crystallization—in the present work the amount of material available was insufficient to permit the writers to proceed further, and it was felt that the isolation of the *d*-form was sufficient proof of the point in question.

Experimental

α-Naphthoquinoline was prepared according to the method of Chalmers, Lions, and Robson (2), boric acid and ferrous sulphate being added, as in the modification of Skraup's quinoline synthesis given by Cohn (3). The naphthoquinoline was oxidized to the acid; yield, about 45%. (The authors wish to acknowledge the assistance of J. N. Robinson of this Department in carrying out the above-mentioned synthesis and some preliminary experiments.)

The general procedure in preparing the salts was as follows. The acid (1.5 gm.) in hot 25% ethanol was added to the calculated amount of the anhydrous alkaloid in hot 95% ethanol (4.00 gm. for quinine and quinidine, 3.63 gm. for cinchonine and cinchonidine). The mixture was filtered, evaporated to crystallization, and the product recrystallized from a suitable solvent.

Quinine salt. Recrystallized three times from 80% ethanol; orthorhombic crystals; yield, 80%. Calcd. for $C_{13}H_9O_4N \cdot 2(C_{20}H_{24}O_2N_2)$: C, 71.3; H, 6.45%. Found: C, 71.1; H, 6.49%.

Quinidine salt. Recrystallized twice from a mixture of ethyl acetate (75%) and ethanol (25%); well formed monoclinic crystals; yield, 80%. Calcd. values as above. Found: C, 71.2; H, 6.36%.

Cinchonine salt. Recrystallized twice from 95% ethanol; small needles; yield, 60%. Calcd. for $C_{13}H_9O_4N \cdot 2(C_{19}H_{22}ON_2)$: C, 73.6; H, 6.43%. Found: C, 73.4; H, 6.51%.

Cinchonidine salt. Recrystallized three times from 25% ethanol; orthorhombic crystals; yield, 87%. Calcd. values as for cinchonine salt. Found: C, 73.9; H, 6.50%.

A detailed crystallographic examination of these salts has been carried out by Dr. M. A. Peacock, Department of Mineralogy, the results to be published elsewhere shortly.

Polarimetric observations. The alkaloid or salt was dissolved in 17 cc. of chloroform, and the rotation measured at 22° C. in a 20 cm. tube.

TABLE II

POLARIMETRIC OBSERVATIONS

Compound	Mol. wt.	Wt. used	α_{5461}	α_{5780}	α_{4358}	$[\alpha]_{5461}$
Quinine	324	0.3629	− 6.1	—	—	−143°
Quinidine	324	0.3570	+10.8	—	—	+258°
Cinchonine	294	0.0626	+ 2.0	—	—	+277°
Cinchonidine	294	0.3602	− 4.0	—	—	− 94°
Quinine salt	891	0.5060	+14.5	+12.5	+28.3	+244°
		0.5151	+14.8	+12.7	+28.9	+244°
Quinidine salt	891	0.5002	−12.1	−10.3	—	−206°
		0.4921	−11.9	−10.2	−23.9	−206°
Cinchonine salt	831	0.5015	−14.8	−12.5	−30.8	−250°
		0.5167	−15.2	−12.8	−31.4	−250°
Cinchonidine salt	831	0.4950	+17.6	+15.0	—	+302°
		0.5031	+17.9	+15.3	+36.5	+303°

Mutarotation experiments. The quinine salt (1.00 gm.) was dissolved in 100 cc. of a mixture of two volumes of ethanol and one volume of chloroform, and the rotation observed in a 40 cm. tube, the first reading being taken three minutes after the salt was wetted. Observed rotations were: $\alpha_{5461} = +3.51°$, $\alpha_{5780} = +3.00°$; this gave $[\alpha]_{5461} = +87.5°$, $[\alpha]_{5780} = +75°$. These values were unchanged after more than 48 hr.

Acid (1.50 gm.) and quinidine (4.00 gm.), both in 95% ethanol, were mixed, the volume was made up to 200 cc., and observed in a 40 cm. tube, the first reading being taken two minutes after mixing; $\alpha_{5780} = -2.66°$. The rotation increased rapidly, and after about one minute of observation had risen to $\alpha_{5780} = -2.91°$, equivalent to $[\alpha]_{5780} = -26.4°$; this value remained unchanged after more than 48 hr. The mutarotation was too rapid to allow accurate and reproducible readings, but its existence could not be doubted.

Experiments similar to the above were carried out with the other salts and similar results obtained. It will be seen that the specific rotations with alcohol present are very much lower than those taken in chloroform—this was also observed with diphenic acid.

Preparation of the Methiodide of the Dimethyl Ester (II)

A portion (10 gm.) of the acid (I) was dissolved in 2000 cc. of distilled water, and the silver salt precipitated by addition of 15 gm. of silver nitrate

and 15 gm. of ammonium acetate in distilled water. The silver salt was dried, refluxed, and shaken for several hours with excess of methyl iodide in ether. The mixture was filtered, the ether removed, and the residual reddish oil heated in a sealed tube with excess of methyl iodide at 100° C. for four hours. The resulting gum crystallized from acetone-ether as yellow crystals; m.p. 149° C.* Recrystallization gave faintly yellow clusters, m.p. 151° C., which remained unchanged after another crystallization. Calcd. for $C_{16}H_{16}O_4NI$: C, 46.5; H, 3.90; I, 30.7%. Found:† C, 46.2, 46.9; H, 3.93, 3.82; I, 30.5%.

A portion (1.80 gm.) of (II) in 60 cc. of 50% methanol was added to 1.92 gm. of silver bromocamphor sulphonate in 30 cc. of distilled water. The mixture was filtered, evaporated *in vacuo*, and the resulting colorless glass crystallized from acetone-ether as small white needles. Recrystallization gave 0.90 gm. of product, m.p. 210° C. Calcd. for $C_{26}H_{30}O_8NSBr$: C, 52.3; H, 5.07; Br, 13.4%. Found:† C, 52.5, 52.5; H, 4.90, 4.79; Br, 13.4%.

A portion (0.500 gm.) of the above-mentioned product in 25 cc. of chloroform in a 20 cm. tube gave $\alpha^{22}_{5461} = +4.28°$, $\alpha_{5780} = +3.75°$, from which $[\alpha]_{5461} = +107°$ and $[M]_{5461} = +637°$; 0.100 gm. in 5 cc. of ethanol gave $\alpha_{5461} = +3.33°$, $\alpha_{5780} = +2.80°$, from which $[\alpha]_{5461} = +83°$, and $[M]_{5461} = +495°$. These and all subsequent rotations were measured in a 20 cm. tube at 22° C.

The solution of the bromocamphor sulphonate in chloroform was shaken with aqueous potassium iodide, the resulting yellow chloroform layer was separated, dried over anhydrous calcium sulphate (Drierite), and the methiodide crystallized by addition of ether; 0.22 gm. was obtained as minute, faintly yellow crystals, m.p. 151° C.

The methiodide (0.0150 gm.) in 5 cc. of chloroform showed rotations of $\alpha_{5461} = +0.94°$, $\alpha_{5780} = +0.73°$, from which $[\alpha]_{5461} = +156.7°$ and $[M]_{5461} = +648°$. Recrystallization of the methiodide gave a product having exactly the same rotation. A portion (0.0150 gm.) in 5 cc. of 95% ethanol showed rotations of $\alpha_{5161} = +0.18°$, $\alpha_{5780} = +0.12°$, and these values were unchanged after the solution was heated at about 70° C. for several hours. From the residues resulting from the above-mentioned experiments, the methiodide recovered gave exactly the same results, and it is apparent that the compound is optically pure.

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* *Melting points are corrected.*

† *Carbon-hydrogen microanalyses by Dr. H. Stantial, Department of Chemistry.*

CONTRIBUTION À L'ÉTUDE D'*ACER SACCHARUM*

II. LA PRÉSENCE D'AMYLASES DANS LA SÈVE D'ÉRABLE ET LES PRODUITS D'HYDROLYSE[1]

PAR ELPHÈGE BOIS[2] ET ARISTIDE NADEAU[3]

Résumé

Les glucides de la sève d'*Acer saccharum* proviennent de l'hydrolyse enzymatique de l'amidon dans les racines. Le seul sucre réducteur présent dans la sève d'érable est le cellobiose, ce qui explique la valeur du pouvoir rotatoire des sirops d'érable avant et après l'interversion. Les amylases de la sève d'érable hydrolysent l'amidon en sucrose et cellobiose, non en maltose, d'où les noms de sucrogène-amylase et cellobiogène-amylase.

Introduction

Bien que les opinions émises sur l'activité des amylases depuis Dubrunfaut, Payen, et Persoz (1830) jusqu'à nos jours, divergent, toutes s'accordent pour affirmer que l'amidon est dédoublé en maltose; seuls Giri et Sreenivasan (4) à notre connaissance, font remarquer que dans le cas de l'amylase de pH optimum 7.0, le point final de la coloration avec l'iode est obtenu sans formation mesurable de maltose. Cette observation démontre, selon ces auteurs, que pendant les premiers stades de la digestion de l'amidon, cette enzyme ne produit pas de groupe réducteur durant les premiers temps de sa présence dans les plantes.

Les travaux faits jusqu'ici sur la sève d'érable avaient surtout pour objet la fabrication du sirop (1, 13), la reconnaissance de sa falsification (6, 7, 11), l'étude de son arome (3, 10, 12) et ses micro-organismes (2).

Jones et Bradlee (8, pp. 137, 138), cependant, ont étudié la teneur de l'érable en hydrates de carbone suivant les saisons et admettent que leur transformation est due à l'activité de certains ferments. Ils ont trouvé, ce que nous avons aussi vérifié, qu'à l'automne, la richesse en sucrose de l'érable, soit dans le tronc au niveau de l'entaille, soit dans les racines, est très faible, tandis que la teneur en amidon est très forte surtout dans les racines. A mesure que la saison avance nous avons constaté un gain en sucrose allant jusqu'à 80% et un autre gain 7 à 8 fois plus grand en sucre réducteur; par contre il y a perte en amidon d'environ 30%.

Partie expérimentale

Cette diminution de l'amidon a été constatée aussi dans l'examen de coupes minces. Ces coupes ont été faites sur des racines fraîchement arrachées, puis mises entre lame et lamelle et traitées uniquement avec une solution d'iode. Pendant l'hiver on constate la présence de nombreux petits grains

[1] *Manuscrit originel reçu le 30 novembre, 1937.*
Contribution du laboratoire de Biochimie, École Supérieure de Chimie, Université Laval, Québec. Contribution basée sur une thèse présentée par Aristide Nadeau pour l'obtention du degré de D.Sc.

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d'amidon dans les vaisseaux. Au temps de la coulée, la plupart des vaisseaux sont vides. Les grains d'amidon ont disparu. Cette transformation est vraisemblablement le résultat de l'activité de certains ferments.

Si on ajoute une solution d'amidon à de la sève d'érable, on constate après quelques heures la disparition de l'amidon ou du moins l'absence de réaction avec l'iode (12). Tout semble se passer comme dans le cas d'une suspension d'amidon en présence de salive, d'extrait de malt ou de taka-diastase. Nous avons étudié systématiquement cette action de la sève sur l'amidon en fonction de la température et du pH.

La cueillette de la sève se faisait au printemps dans des fioles contenant une quantité suffisante de toluène pour empêcher tout développement microbien; elle était conservée dans la glacière.

Influence de la température

On détermine la vitesse d'hydrolyse d'une solution d'amidon en présence de sève d'érable à différentes températures. L'expérience est faite au moyen d'une solution d'amidon à 1% et d'une quantité égale de sève tamponnée à pH 6.6 à 6.8 moyen de solutions $M/15$ de phosphates monopotassique et disodique. Le pH désiré est obtenu par un mélange de l'une et de l'autre

TABLEAU I

HYDROLYSE DE L'AMIDON À PH CONSTANT (6.6 À 6.8)

T, °C.	Après 17 h.	Après 24 h.	Après 40 h.	Après 48 h.
2	Violet	Rouge violet	Rouge brun	Rouge brun
8	Rouge violet	Rouge brun	Brun rouge	Brun rouge
15	Rouge brun	Brun rouge	Brun	Brun clair
20	Rouge brun	Brun	–	–
40	Brun	–	–	–
50	Brun	–	–	–
60	Brun rouge	Brun	Brun clair	–

solution en proportion déterminée. Au bout d'intervalles de temps fixes, on prélève 2 cc. de la solution à laquelle on ajoute cinq gouttes d'une solution d'iode $N/50$. La coloration obtenue indique la plus ou moins grande hydrolyse de l'amidon.

A 2° C., tableau I, on constate une coloration rouge brun même après 48 h. Pour le même temps à 8° C. la coloration est brune, tandis qu'à 15° C. elle est d'un brun clair. A 20° C. aucune coloration avec l'iode après 40 h. A 40° et 50° C. moins de 24 h. suffisent pour une réaction négative. A 60° C. légère coloration brune après 40 h. Si la solution est portée à 80° C. pendant quelque temps, il n'y a plus d'hydrolyse; la coloration bleue persiste; les ferments sont détruits à cette température. Les températures entre 40° à 50° C. semblent donc être les plus favorables.

Influence du pH

Les mêmes solutions d'amidon et de ferments maintenues à 40° C. sont tamponnées cette fois-ci à differents pH à partir de 4.0 à 7.5 au moyen de

mélanges en différentes proportions de solutions $M/15$ de phosphates monopotassique et disodique. Les résultats obtenus sont indiqués dans le tableau II. Comme on le voit à pH 4.2 une coloration rouge brun persiste après 48 h.; de pH 5.5 à pH 6.75, moins de 20 h. suffisent pour une réaction négative, tandis qu'il faut un peu plus de 48 h. à pH 7.5.

Des résultats sensiblement les mêmes sont obtenus à 50° C. (tableau III). La zone de pH la plus favorable s'étend comme dans le premier cas de pH 5.2 à pH 6.7. Cette température semble être moins favorable pour les pH extrêmes. Ces faits suffisent pour prouver sans aucun doute la présence des ferments du amylase dans la sève d'érable.

TABLEAU II

HYDROLYSE DE L'AMIDON À TEMPÉRATURE CONSTANTE (40° C).

pH moyen	Après 17 h.	Après 24 h.	Après 40 h.	Après 48 h.
4.20	Rouge brun	Rouge brun	Rouge brun	Rouge brun
4.79	Brun	Brun	–	–
5.38	Brun	–	–	–
5.77	Brun	–	–	–
6.16	Brun	–	–	–
6.75	Brun	–	–	–
7.46	Brun rouge	Brun	Brun	Brun clair

TABLEAU III

HYDROLYSE DE L'AMIDON À TEMPÉRATURE CONSTANTE (50° C.)

pH moyen	Après 17 h.	Après 24 h.	Après 40 h.	Après 48 h.
4.03	Violet	Rouge brun	Rouge brun	Rouge brun
4.84	Brun rouge	Brun	Brun	Brun clair
5.20	Brun	–	–	–
5.81	Brun	–	–	–
6.17	Brun	–	–	–
6.67	Brun	–	–	–
7.42	Rouge brun	Brun rouge	Brun rouge	Brun

Les produits d'hydrolyse

Poussant plus avant nos recherches, nous avons voulu caractériser le ou les produits formés par le dédoublement de l'amidon. Puisque les glucides de réserve se trouvent emmagasinés dans les racines et que durant l'hiver nous voyions disparaître l'amidon de ces racines, nous avons cru y trouver ses produits de transformation. D'autre part on constate aussi dans le dosage des sucres du sirop d'érable qu'il y a une faible quantité de sucre réducteur qui ne peut pas provenir de l'interversion du sucrose. Nous avons d'ailleurs trouvé que tout échantillon de sève d'érable, malgré les précautions prises lors de la récolte pour empêcher l'interversion du sucrose, possédait un pouvoir réducteur supérieur au pouvoir réducteur d'une solution de sucrose de même concentration. Certains admettent la présence de lévulose (1); nous avons donc recherché la nature de ce produit réducteur.

Cellobiose

(*a*) *Dans les racines.* Nos études ont porté d'abord sur les racines. Débarrassées complètement de leur écorce, elles étaient réduites en pulpe. On traite 100 g. de cette pulpe par deux litres d'alcool éthylique à 80% pendant 30 min. à l'ébullition en présence de carbonate de calcium pour neutraliser les acides. Après filtration et distillation de la solution alcoolique, le résidu est repris par l'eau, et décoloré au noir animal. On ajoute 100 cc. de la solution obtenue, contenant 3% de solide environ, à 5 g. de chlorhydrate de phénylhydrazine purifié et 10 g. d'acétate de sodium. On chauffe le tout au bain-marie pendant une heure. Il y a formation d'une osazone à chaud. Après l'avoir filtrée et purifiée, on détermine sa forme cristalline et sa solubilité comme glucosazone (P.F., 205° C.). Par refroidissement le filtrat laisse déposer une osazone jaune brun, cristallisée en aiguilles groupées en forme d'oursin. Cette osazone purifiée par recristallisation dans l'alcool a un point de fusion de 198° C.

(*b*) *Dans la sève.* La même methode employée avec de la sève fraîche donne des résultats semblables. Avec 250 cc. de sève fraîche, chauffée au bain-marie pendant une heure en présence de 7 g. de chlorhydrate de phénylhydrazine et 14 g. d'acétate de sodium, il y a formation tantôt des deux mêmes osazones, tantôt uniquement de l'osazone cristallisée en aiguilles groupées en forme d'oursin. Le glucose, présent dans certains cas, peut provenir de l'hydrolyse du sucrose ou du cellobiose.

(*c*) *Amidon en présence de sève.* Les mêmes résultats sont obtenus avec de la sève en présence d'amidon. La sève est dialysée dans des sacs de cellophane jusqu'à disparition des sucres. Deux cents centimètres cubes de sève ainsi traitée et une quantité égale d'une solution d'amidon à 1% sont tamponnées à pH 6.5 au moyen de tampon aux phosphates comme plus haut. Le tout est placé à 37° C. jusqu'à disparition de la coloration avec l'iode. La solution est alors filtrée, puis additionnée de 10 g. de chlorydrate de phénylhydrazine et de 20 g. d'acétate de sodium. Les osazones obtenues dans les conditions mentionnées plus haut, sont identiques à celles trouvées précédemment. Nous faisons remarquer que dans les trois cas nous n'avons jamais obtenu la maltosazone.

Le dosage de l'azote par la méthode Kjeldahl sur cette osazone cristallisée en aiguilles groupées en forme d'oursin donne 10.6% d'azote (valeur théorique, 10.77%), ce qui prouve la présence d'un sucre réducteur en C_{12}. De plus l'osazone obtenue à partir d'une solution de cellobiose authentique, a donné la même forme crystalline, la même solubilité et le même point de fusion (198° C.). Le mélange des deux osazones donne le même point de fusion. Le sucre réducteur formé est bien du cellobiose.

2. *Sucrose*

La présence de sucrose dans la sève nous a conduits à chercher s'il n'y avait pas formation de ce sucre dans l'hydrolyse de l'amidon par les ferments de la sève d'érable. Une solution d'amidon à 1% et de la sève dialysée sont

tamponnées à pH 6.6 au moyen de tampons aux phosphates et laissées à 17° à 18° C. environ, jusqu'à l'absence de réaction avec l'iode. Le dosage des sucres est fait par la méthode Munson et Walker, avant et après traitement par l'invertase, au début et à la fin de l'hydrolyse. Une augmentation en sucre correspondant à 30% de la quantité théorique de formation de sucrose est alors constatée. Comme l'action de l'invertase employée était nulle sur une solution de cellobiose et aussi sur la solution d'amidon, cette augmentation ne peut être attribuée qu'à la transformation de l'amidon en sucrose.

Discussions et conclusions

1. Nos travaux permettent de corriger et de développer, en partie du moins, la théorie de la génèse du sucre d'érable. Chez *Acer saccharum*, les glucides résultant de la fonction chlorophyllienne au niveau des feuilles pendant l'été, sont transportées au fur et à mesure de leur production dans les racines, où ils se transforment en amidon. Tous les vaisseaux libériens et les rayons médullaires en sont gonflés. Durant l'hiver, suivant les variations de la température, cet amidon s'hydrolyse en faible quantité, puis au printemps le phénomène s'accentue brusquement et l'amidon disparaît pour donner naissance à deux glucides en C_{12}: le sucrose et le cellobiose, que nous trouvons dans la sève au moment de la coulée.

2. Le seul sucre réducteur présent dans la sève au moment de sa sortie de l'érable est le cellobiose.

Déjà en 1911, Bryan (1, p. 64) luttait contre l'affirmation de la présence de sucre interverti dans la sève d'érable, qu'on expliquait alors par une hydrolyse du sucrose par les cellules de l'arbre qui devaient y trouver un combustible plus apte à être utilisé.

Bryan mettait aussi en doute une deuxième hypothèse basée sur les différences entre les dosages chimiques et polarimétriques des glucides de la sève. En effet, on constatait que la valeur du pouvoir rotatoire, avant comme après l'interversion, était toujours inférieure à ce qu'elle aurait dû être si l'on n'avait eu affaire qu'au sucrose. L'on admettait alors la présence de lévulose dont le pouvoir rotatoire $[\alpha]_D = -92°$.

Après bien des mesures et des dosages, Bryan concluait que si le lévulose est présent, il doit tout de même être accompagné d'une autre substance optiquement active.

Avec le cellobiose, nous avons une explication de ces lectures trop faibles au polarimètre. Nous sommes en présence de sucrose, $[\alpha]_D = +66°$, et de cellobiose, $[\alpha]_D = +35°$. Donc avant l'interversion la lecture est plus faible que pour une solution de sucrose de même concentration. Après l'hydrolyse, nous avons: sucre interverti $[\alpha]_D = -19.84°$, et le glucose, $[\alpha]_D = +52°$ provenant du cellobiose, la lecture est encore inférieure à celle d'une solution de sucre interverti seul. La preuve que nous n'avons que du cellobiose comme sucre réducteur, découle de l'obtention de son unique phénylosazone caractérisée par son point de fusion mixte, sa teneur en azote, sa cristallisation et sa solubilité.

3. Il y a dans la sève d'*Acer saccharum* deux ferments du type amylase. Les premiers qui aient signalé l'hydrolyse de l'amidon par la sève d'érable n'ont donné aucune preuve que le phénomène était enzymatique.

Nous apportons ces preuves: un chauffage de la sève à 80° C. fait disparaître ses propriétés amylolytiques, la dialyse de la sève amène une diminution de ces propriétés par séparation du ou des co-ferments. L'action de la sève sur l'amidon, contrôlée par la réaction avec l'iode, varie suivant la température et la concentration en ions hydrogène. Elle est plus marquée à 40° à 50° C. et dans la zone de pH compris entre 5.3 à 6.7.

4. Ces ferments hydrolysent, *in vitro*, une solution d'amidon en cellobiose et sucrose sans production de maltose. C'est croyons-nous, la première fois que ce fait est observé et étudié.

Cette conclusion est des plus importantes. Il était admis, d'une part (Haworth (5)) que la caractéristique de l'amidon, c'était son hydrolyse en maltose, tandis que celle de la cellulose c'était son hydrolyse en cellobiose. L'étude de ces deux polysaccharides et de leurs dérivées immédiats était pour ainsi dire, basée sur cette admission. D'après les résultats obtenus ici, il faudrait modifier les hypothèses émises jusqu'à aujourd'hui sur la constitution de l'amidon et admettre que les cycles pyraniques et furaniques coexistent dans la chaîne d'amidon.

D'autre part, il était aussi reconnu que dans les feuilles des plantes, il y a, durant le jour, par ordre d'importance, du sucrose, des hexoses, et de l'amidon. Durant la nuit la quantité de l'amidon croît aux dépens des autres glucides. On admettait donc la formation des hydrates de carbone d'après cette suite de phénomènes.

$$CO_2 + H_2O \longrightarrow HCOH$$

$$\text{Amidon} \longleftarrow C_6H_{12}O_6 \underset{\text{Nuit}}{\overset{\text{Jour}}{\rightleftharpoons}} \text{Sucrose}$$

Mais cet amidon disparaissait de quelle façon? "In none of the starch-bearing (Miller (9, p. 436)) leaves were they able to detect maltose, . . ." (9, p. 440) ". . . since so far as anyone knows, cane sugar does not arise directly from starch."

Nous avons démontré la transformation de l'amidon en sucrose, *in vitro*; nous l'avons constaté chez les racines de l'érable et nous croyons que dans les feuilles il en est de même.

5. Les enzymes contenues dans la sève d'*Acer saccharum* qui hydrolysent l'amidon en sucrose et cellobiose; nous les avons nommées: sucrogène-amylase et cellobiogène-amylase. Elles se distinguent nettement des amylases connues à date par les produits formés.

Le prénom "sucrogène" indique bien ici le phénomène hydrolytique observé, et nous l'employons par opposition à saccharogène-amylase déjà connu, qui désigne le ferment hydrolysant l'amidon en un sucre en C_{12} (maltose) tandis que le dextrinogène-amylase indique une hydrolyse partielle de l'amidon.

La sucrogène-amylase se rapproche du type β-amylase parce qu'au voisinage de la température optimum, la coloration avec l'iode persiste longtemps. La cellobiogène-amylase semble appartenir au type α-amylase.

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CONTRIBUTION À L'ÉTUDE D'*ACER SACCHARUM*

III. ACTIVITÉ DES AMYLASES DE LA SÈVE D'ÉRABLE[1]

PAR ELPHÈGE BOIS[2] ET ARISTIDE NADEAU[3]

Résumé

Les conditions optima de l'activité de la sucrogène-amylase sont: un pH de 6.6 et une température de 8° C.; tandis que la cellobiogène-amylase a son maximum d'action à 50° C. et à pH 4.8.

L'activité de ces amylases augmente à mesure que la saison de coulée avance. Au printemps, le pH de la sève est précisément le pH optimum de formation du sucrose (pH 6.6). De plus la température de cette saison (0° à 15° C.) est favorable à la formation du sucrose qui cesse avec la venue des jours chauds.

Introduction

Après avoir reconnu l'existence d'amylases dans la sève d'érable et avoir caractérisé les glucides résultants de leur action sur l'amidon, nous sommes en mesure de poursuivre l'étude de ces ferments et de déterminer dans quelles conditions leur travail est effectué. Nous pourrons ainsi vérifier ce que nous avons déjà avancé (2) au sujet du maximum d'activité, qui, d'après les travaux de Sherman, Thomas et Caldwell (14), doit-être voisin du minimum de pouvoir tampon analogue au point isoélectrique.

La mesure de l'activité se fera en déterminant à intervalles fixes, les quantités de sucre réducteur (cellobiose) et de sucrose produites au cours de l'hydrolyse d'une solution d'amidon par la sève d'érable en faisant varier la température et le pH.

Ce sont les deux facteurs les mieux étudiés à date. Ils font l'objet d'un grand nombre de travaux dont les conclusions sont plus ou moins contradictoires. Il est juste de dire avec Kopaczewski (4) que l'activité des amylases est influencée par la pureté et l'âge des enzymes, par la composition et l'état physique du substrat, par les conditions expérimentales et par la concentration des cations ou anions présents à côté de l'ion H^+; autant de facteurs qui peuvent faire varier les résultats.

Partie expérimentale

A. TECHNIQUE DES MANIPULATIONS

Préparation de la solution d'amidon

L'amidon soluble pesé est délayé dans un peu d'eau chaude. L'ébullition est continuée pendant deux minutes, puis le tout est refroidi aussi rapidement que possible. Le volume d'eau est ensuite ajusté de manière que la solution soit de concentration égale à 1%.

[1] *Manuscrit reçu le 30 novembre, 1937.*
Contribution du laboratoire de Biochimie, École Supérieure de Chimie, Université Laval, Québec.
Contribution basée sur une thèse présentée par Aristide Nadeau pour l'obtention du degré de D.Sc.

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Les ferments

La sève cueillie au temps de la coulée (mars, avril) contient, en plus des ferments, une quantité de sucrose qui varie de 1 à 4% et quelques centièmes de un pour cent de cellobiose.

Les essais d'élimination de ces glucides (précipitation, dialyse, ultrafiltration) nous ont toujours laissé une solution de ferments de faible activité.

C'est donc avec de la sève telle qu'obtenue de l'arbre que nous avons fait nos expériences. Pour la conserver, elle était mise en bouteille avec une quantité suffisante de toluène pour la saturer et maintenue dans un endroit frais, environ 12° C. Ce mode de conservation est efficace pour une période de quelques mois. Les manipulations qui ont servi à l'obtention des courbes 1-2-3 ont été faites à la fin de l'été avec cette sève.

Pour les périodes de conservation plus longues (8 à 12 mois), la sève fraîche était concentrée dans le vide, à une température inférieure à 25° C., jusqu'à une teneur de 60% en solide. Ce sirop était ensuite saturé de toluène et conservé à la glacière. Pour l'emploi, il suffisait de le diluer jusqu'à la concentration de la sève originale, c'est-à-dire 4 à 5% de solide, déterminée au moyen du réfractomètre.

Le pouvoir amylolytique pouvait se maintenir de cette façon, tandis que chez la sève non concentrée, il diminuait, déjà de 50% après huit mois. Nous avons pu contrôler que cette perte était proportionnelle à la transformation du sucrose contenu dans la sève en sucre interverti; les sels présents favorisaient cette interversion.

Le sucre interverti jouerait vis-à-vis de la sucrogène-amylase et la cellobiogène-amylase le rôle d'inhibiteur, tout comme il a été établi pour le β-maltose vis-à-vis les β-amylases (16, p. 138).

De plus, dans la sève concentrée dans le vide, dialysée jusqu'à la disparition du sucrose et du sucre réducteur, tamponnée à pH 6.8, et saturée de toluène, nous avons constaté l'apparition du sucre réducteur, après un séjour de 10 mois à la glacière. Ce qui indique la présence dans la sève d'érable de glucides supérieures, non dialysables, intermédiaires de l'amidon, qu'on est convenu d'appeler encore des achroodextrines.

La sève uniquement concentrée dans le vide s'est par contre toujours montrée stable.

Les tampons

Les solutions tampons servant à fixer le pH des liquides à hydrolyser, étaient préparées au moyen de quantités variables de solution $M/15$ de phosphate disodique, phosphate monopotassique et d'acide phosphorique. Le pH était determiné à l'électrode à la quinhydrone au début et à la fin de l'hydrolyse sur chaque liqueur à l'étude. Les pH se sont maintenus à peu près constants (± 0.05) tout le temps de l'hydrolyse.

La température

Pour les températures de 60° C. à 4 0° C., nous avions recours à un bain d'eau muni d'un agitateur et d'un thermostat qui permettaient de régler et

de maintenir la température à $\pm 0.5°$; un système de refrigération par circulation d'eau froide était ajusté pour la température de 16° C., tandis que nous avons aménagé des compartiments dans la glacière pour les séries d'expériences faites à 8° C. et à 2° C.

Les témoins

La présence de cellobiose, de sucrose et de dextrine dans la sève servant d'agent d'hydrolyse, nous posait tout un problème, à savoir: quelles seraient nos expériences témoins? Nous ne prétendons pas l'avoir tranché d'une façon précise, mais nous croyons avoir établi une marge de sécurité suffisante pour les conclusions que nous voulons tirer de ce travail.

Une étude des modifications des glucides de la sève suivant les variations de la température et du pH, nous a permis de fixer un maximum entre les pH 6.5 et 6.8. Comme l'augmentation en sucre ne dépassait pas 10 mg. dans 25 cc. de solution, il n'y a pas eu de corrections de faites pour cette augmentation dans les graphiques qui suivent. Par mesure de précaution, pour chaque température étudiée, un essai témoin a été fait où le volume d'amidon était remplacé par une quantité égale d'eau. C'est le témoin compagnon.

Nous avions en plus les témoins au temps zéro, ce qui nous permettait d'apprécier l'influence des mélanges de sève, d'amidon et de tampon sur nos déterminations quantitatives des sucres formés.

Les dosages

La méthode de Bang (7, p. 1189) au sulfocyanure cuivrique, employée par plusieurs, Ohlsson et ses collaborateurs en particulier (9, 10, 11, 12), nous a servi au début et les résultats s'accordent suffisamment avec ceux que nous publions. La teinte du virage nous semblait d'appréciation délicate avec le changement d'éclairage; par ailleurs, la sève d'érable contenant une quantité appréciable de sucrose, 3 à 4%, cette microméthode était débordée lorsqu'il s'agissait de doser le sucre total.

Nous avons ensuite préféré augmenter le volume des liqueurs en expérience et nous avons adopté pour le dosage du sucre réducteur (cellobiose) et du sucrose, la méthode Munson et Walker (A.O.A.C.). La défécation est faite par addition d'une suspension d'alumine, l'interversion du sucrose par une solution d'invertase d'activité standard tamponnée à pH 4.5 au moyen d'acide acétique $N/5$.

Les quantités de glucides (cellobiose en présence de sucrose et sucrose ou mieux sucre interverti en présence de cellobiose) correspondant au poids du cuivre trouvé, étaient déterminées au moyen de deux courbes: (*a*) l'une obtenue avec une solution de sucrose et de cellobiose de concentration bien déterminée (1%; c'était en moyenne la teneur de nos liqueurs à doser). Le dosage du sucre réducteur était fait sur les quantités croissantes de cette solution; (*b*) l'autre, avec les mêmes quantités, mais après traitement par l'invertase. Tous nos dosages, soit pour établir nos courbes, soit pour déterminer les quantités de sucre formé au cours de l'hydrolyse, soit pour les témoins, étaient donc faits dans des conditions identiques.

Calcul des dosages

Dans un premier dosage: l'amidon + sève + tampon, au temps zéro, on obtient une quantité de sucre réducteur:

R_1 = sucre interverti + sucre réducteur contenu dans l'amidon + cellobiose de la sève.

Après l'action de l'invertase on obtient:

$$R_2 = R_1 + \text{sucrose de la sève.}$$
$$R_2 - R_1 = S_1, \text{ sucrose de la sève.}$$

Après 17, 24, 40, et 48 h. d'hydrolyse on dose une quantité de sucre

$$R_3 = R_1 + \text{cellobiose venant de l'amidon}$$
$$\text{d'où } R_3 - R_1 = R_5, \textit{ cellobiose formé.}$$

Après l'action de l'invertase on obtient:

$$R_4 = R_2 + R_5 + \text{sucrose venant de l'amidon}$$
$$\text{d'où } R_4 - R_2 - R_5 = S_2, \textit{ sucrose formé.}$$
$$\text{ou } S_2 = R_4 - R_2 - R_3 + R_1.$$

Les graphiques qui suivent indiquent la quantité en milligrammes de sucre réducteur (cellobiose (R_5)) ou de sucrose (S_2) formé dans 25 cc. de solution, à une température déterminée et en fonction du pH. Chaque courbe indique la quantité de sucre produit aux différentes étapes de l'hydrolyse: 17, 24, 40, 48, ou 65 h.

L'hydrolyse

Les mélanges hydrolytiques étaient constitués comme suit:

1 volume de la solution d'amidon 1%, saturée de toluène.
1 volume de sève d'érable, saturée de toluène.
0.25 volume de la solution tampon.

Ils étaient faits dans des bouteilles bouchées à l'émeri. La concentration en amidon était donc de 0.44%.

B. Résultats

1. Les essais préliminaires nous ont permis d'observer qu'aux températures supérieures à 65° C. la sève d'érable n'amenait qu'une très faible hydrolyse de l'amidon et lorsqu'elle était maintenue 10 min. à 70° C., elle perdait toute activité; on admet alors la destruction des enzymes.

Les résultats que nous présentons des experiences faites à 60°, 50°, et 40° C. forment un groupe homogène, c'est-à-dire que la solution d'amidon et de la sève employée étaient les mêmes pour toute la série des manipulations.

Nous remarquons en premier lieu que la formation de sucrose est nulle à ces températures, du moins elle n'est pas appréciable dans des conditions où nous avons dû opérer. De plus, le sucrose présent dans la sève subit une interversion d'autant plus forte que la température est plus élevée. Les témoins nous l'indiquent clairement. Nous savons d'après Bertrand et Holderer (1) que 72 mg. de cuivre correspondent à 51.8 mg. de cellobiose, aussi à 37 mg. de sucre interverti ou 35.15 mg. de sucrose.

Or à 60° C., la plus forte quantité de sucre réducteur (exprimé en cellobiose) du témoin est 12.7 mg., ce qui représenterait 8.63 mg. de sucrose contenu dans le mélange au début de l'hydrolyse. A 50° C. on obtient 6.6 mg. ce qui correspond à 4.5 mg. de sucrose, et à 40° C., 3.2 mg. correspondant à 2.2 mg. de sucrose.

Ce sucre représente au plus 0.1 de l'augmentation du pouvoir réducteur de la solution à l'étude. Nous pouvons donc admettre que nos courbes, tout en n'étant pas exactes doivent indiquer l'allure générale du phénomène.

A 60° C. (Fig. 1), la formation du sucre réducteur, cellobiose passe par un maximum à pH 4.8 (33 mg. dans 25 cc. après 48 h. équivalent à 30% de l'amidon mis à hydrolyser). L'activité diminue avec l'augmentation de la valeur du pH.

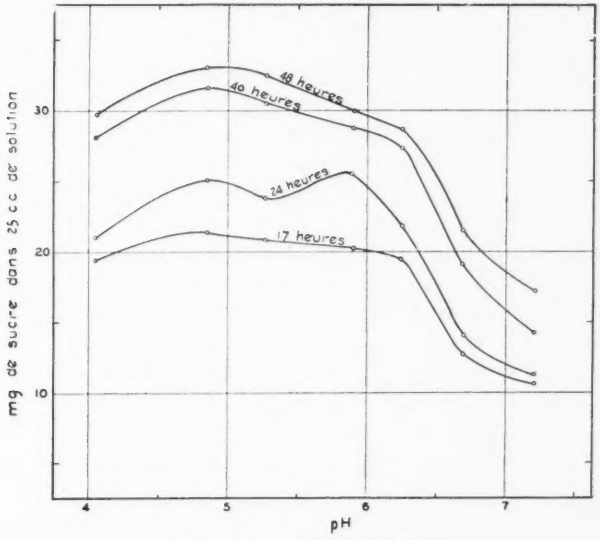

FIG. 1. *Sucre réducteur à 60° C.*

A 50° C. (Fig. 2) nous avons deux maxima, le premier à pH 4.8 avec une production de cellobiose égale à 35.4% de l'amidon, et un second à pH 6.6 où la quantité de sucre réducteur représente 45.4% de l'amidon.

Ce second maximum ne se retrouve pas chez les autres courbes. Nous avons répété l'hydrolyse dans les mêmes conditions avec un autre échantillon de sève et une nouvelle solution d'amidon. Nous avons constaté la même anomalie, c'est-à-dire un second maximum à pH 6.6, mais inférieur cette fois au premier maximum à pH 4.8 pour les courbes après 40 et 48 h. d'hydrolyse, et égal au premier pour celles après 17 et 24 h. d'hydrolyse. Nous reconnaissons être dans la zone critique où plusieurs facteurs entrent en jeu, nous le verrons plus loin.

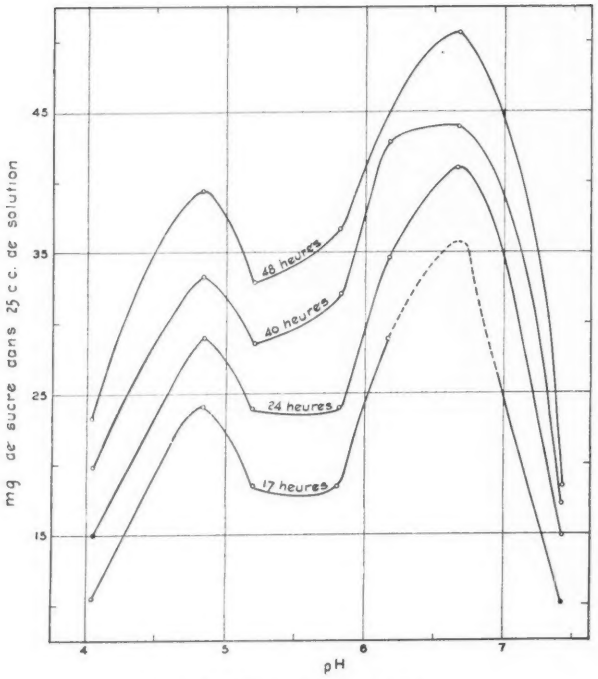

FIG. 2. *Sucre réducteur à 50° C.*

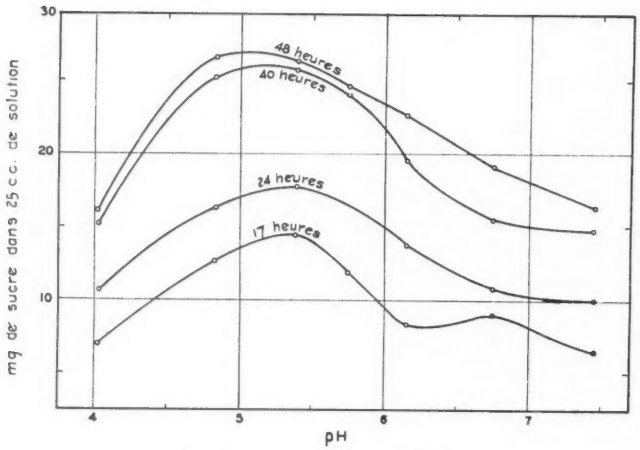

FIG. 3. *Sucre réducteur à 40° C.*

A 40° C. (Fig. 3) la quantité de sucre réducteur formé ne dépasse pas 26.6 mg. dans 25 cc. de l'hydrolysat, ce qui équivaut à la transformation de 24.1% d'amidon. A cette température nous remarquons que le pH optimum oscille entre 4.8 et 5.3.

2. Nous ne mentionnerons pas les expériences faites entre 40° et 16° C., elles sont d'intérêt tout à faire secondaire, puisque nous passons par une étape intermédiaire des activités des deux amylases. Nous retrouvons d'une manière plus accentuée dans les courbes qui suivent, les mêmes phenomènes: diminution de l'activité de la cellobiogène-amylase et apparition de l'activité de la sucrogène-amylase.

Les hydrolyses faites à 16°, 8°, et 2° C. forment un second groupe homogène. A 16° C. (Fig. 4) la formation de sucre réducteur ne représente plus que 17.7% de l'amidon (19.5 mg.) à pH 4.8 le plus favorable; tandis qu'aux pH extrêmes, la quantité de cellobiose est très faible, inférieure à 10 mg.

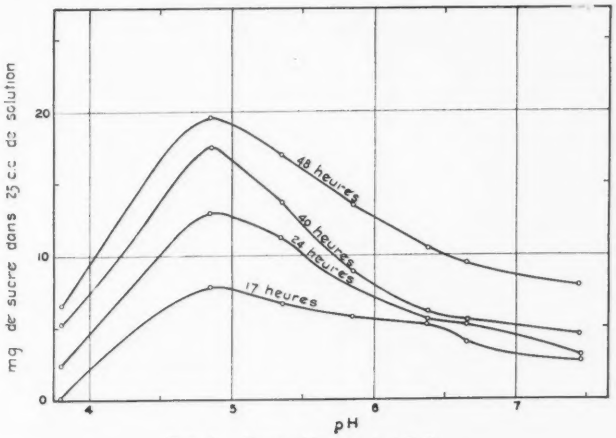

FIG. 4. *Sucre réducteur à 16° C.*

La production du sucrose (Fig. 5) ne fait plus de doute, le maximum s'établit à pH 6.6 avec 32.4 mg. ce qui indique une hydrolyse de l'amidon égale à 29%. Un total de (29 + 17.7 = 46.7%).

A 8° C. (Fig. 6) la cellobiogène-amylase devient moins active, le pH optimum prend une valeur plus élevée, 5.4 au lieu de 4.8, et la quantité de sucre réducteur formé ne représente que 13% de l'amidon.

Pour le sucrose (Fig. 7), cette température s'est révélée la plus favorable, la quantité de sucre augmente légèrement aux pH les plus bas, puis s'accentue considérablement pour marquer un maximum à pH 6.6. La proportion d'amidon ici transformée en sucrose est de 40%; l'hydrolyse totale (40 + 13 = 53%).

Notons encore que dans les conditions les plus favorables, même après 65 h. d'hydrolyse, il reste une quantité d'amidon non transformé que nous

évaluons à 30%. C'est la partie moins soluble, non colorable par l'iode que l'on est convenu d'appeler "amylopectine".

De plus les témoins compagnons de 24 h. et de 65 h., soit pour le sucre réducteur ou le sucrose, sont les mêmes, indice que du côté de la sève la réaction est complète.

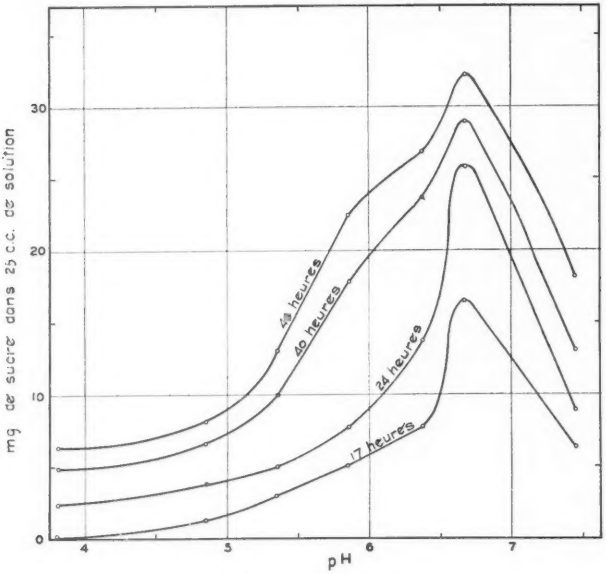

FIG. 5. *Sucrose à 16° C.*

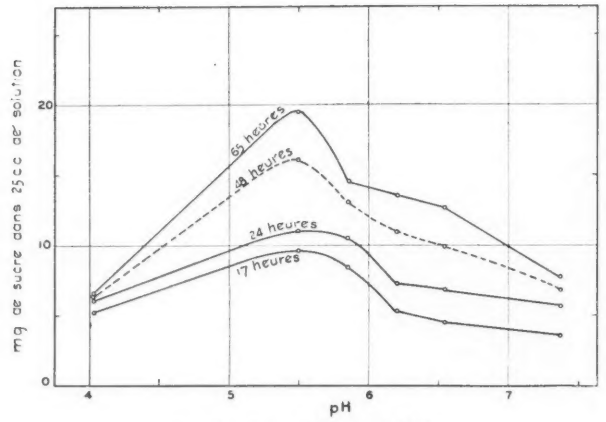

FIG. 6. *Sucre réducteur à 8° C.*

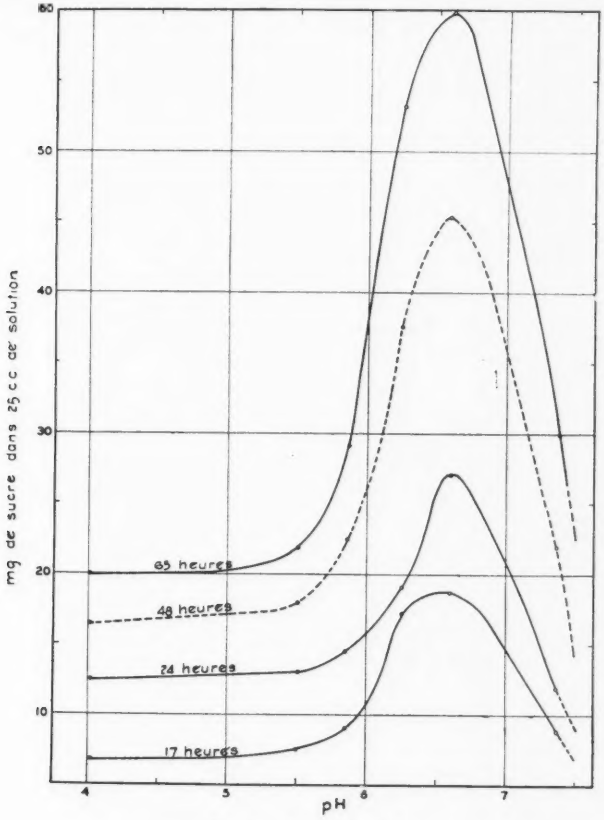

FIG. 7. *Sucrose à 8° C.*

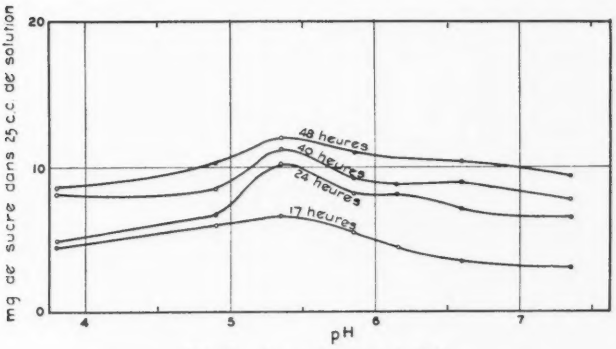

FIG. 8. *Sucre réducteur à 2° C.*

A 2° C. (Fig. 8) le cellobiose formé n'équivaut plus qu'à 10% de l'amidon à pH 5.35. L'activité de la cellobiogène-amylase est de nouveau déplacée vers un pH plus élevé.

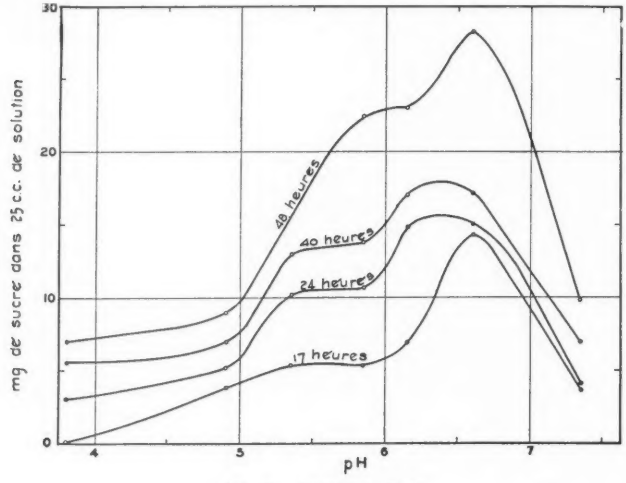

FIG. 9. *Sucrose à 2° C.*

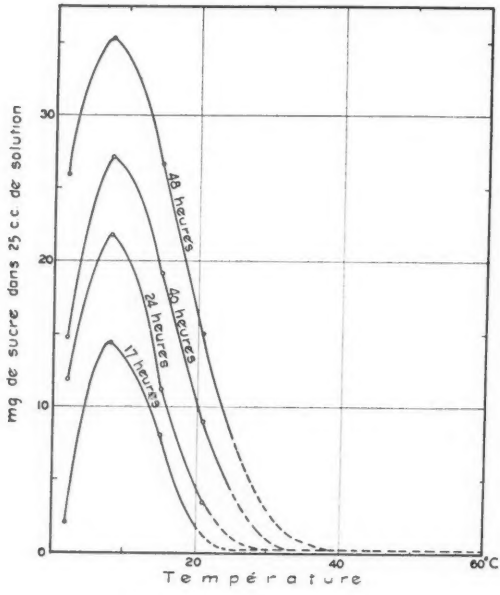

FIG. 10. *Sucrose à pH 6.62.*

La formation de sucrose (Fig. 9) est encore importante; à pH 6.6, 28.3 mg. après 48 h. ou 23.6% de l'amidon transformé en sucrose. Il restait sans doute de l'amidon intact puisque la réaction avec l'iode était encore positive au moment du dernier dosage.

Chez l'érable

Certaines constatations faites au printemps sur la sève fraîche, apportent quelques faits nouveaux qui viennent confirmer les résultats déjà obtenus. Chaque jour, le pH a été déterminé au moyen de l'électrode à la quinhydrone. La sève, provenant toujours d'une même entaille, a un pH qui se maintient aux environs de 6.6. La présence de pluie ou de neige a pour effet d'augmenter le pH. Il atteint parfois 7.4, surtout à la fin de la saison quand l'entaille vieillit et que la température devient plus chaude. Mais la sève revient à son pH normal (pH 6.6) si une nouvelle entaille est faite.

Nous avons donc entrepris un troisième groupe de déterminations qui pourront nous donner quelques renseignements sur les phénomènes dont l'*Acer saccharum* est le siège au printemps.

Un même mélange d'amidon et de sève, tamponné à pH 6.62, a été mis à hydrolyser à différentes températures. Les résultats font l'objet des courbes Figs. 10 et 11. Les maxima à 8° C. pour la formation de sucrose et à 50° C.

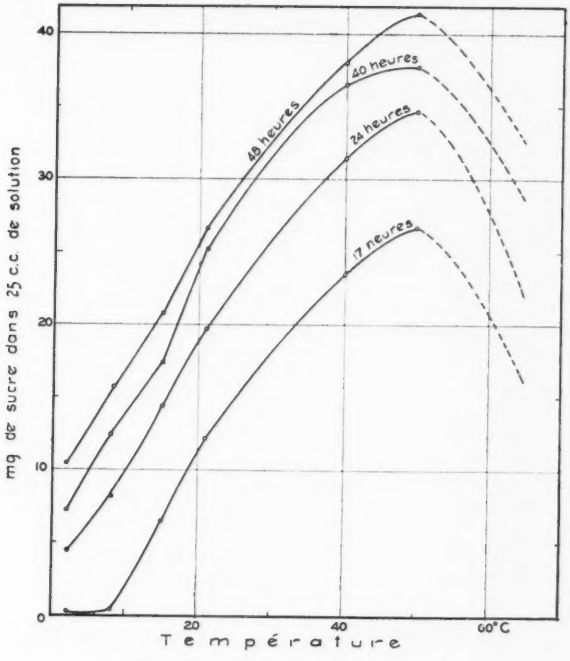

FIG. 11. *Sucre réducteur à pH 6.62.*

pour celle du sucre réducteur, le cellobiose, confirment bien ce que nous venons de présenter, et nous font voir que l'élaboration de sucrose l'emporte du beaucoup sur celle du cellobiose aux mois de mars et d'avril.

C'est en effet lorsque la température oscille autour de 8° C. (45° F.) que l'écoulement de la sève est le plus abondant et il s'arrête avec la venue des jours chauds.

Nous avons aussi mesuré la vitesse d'hydrolyse d'une solution d'amidon en présence de sève pendant tout le temps de la coulée. Chaque jour, une série de tubes contenant 2 cc. de sève fraîchement cueillie, 1 cc. d'amidon soluble et 1 cc. de tampon aux phosphates (pH 6.6) le tout saturé de toluène, sont placés à 37° C.

Au début du printemps, il faut un séjour de 40 h. environ à cette température pour que la réaction avec l'iode soit négative; au milieu de la saison 24 h. suffisent et à la fin, 20 h. La quantité de ferments dans la sève croît donc à mesure que la saison de coulée progresse.

Discussions et conclusions

1. D'après nos recherches, les conditions optima de l'activité de la sucrogène-amylase sont: pH 6.6 et une température de 8° C.

Pour la cellobiogène-amylase nous ne pouvons pas être aussi catégorique pour le moment, les courbes obtenues aux températures supérieures à 40° C., nous indiquent bien que le pH optimum est 4.8 et qu'il se déplace vers pH 5.3 à mesure que la température baisse, mais à 50° C. nous sommes en présence d'un second maximum à pH 6.6 qui semble être le résultat d'un ou de plusieurs phénomènes secondaires. Soit interversion du sucrose à mesure de sa formation (1 mg. de sucre interverti = 2.66 mg. Cu), soit l'hydrolyse du cellobiose en deux glucoses (1 mg. de glucose = 2.04 mg. Cu; tandis que 1 mg. de cellobiose = 1.38 mg. Cu). Peut-être encore s'agit-il d'une modification d'un des constituants de l'amidon, l'amylopectine par exemple.

2. Le pH optimum de formation du sucrose, le pH 6.6, est dans la zône d'un des minima de pouvoir tampon situé à pH 6.5 à 6.7. L'autre minimum à pH 4.6 à 4.9 correspond assez bien à pH optimum compris entre 4.8 et 5.4 de formation de sucre réducteur (2).

Ces faits confirment les résultats de Sherman, Thomas, et Caldwell (14) à savoir; l'activité maximum des ferments se trouve au voisinage du point isoélectrique.

3. Les solutions ou suspensions d'amidon telles que préparées couramment ne sont pas entièrement hydrolysées par les deux amylases contenues dans la sève d'érable.

Les autres amylases agissent d'une façon analogue. Le pourcentage de la partie réfractaire varie avec le mode de préparation et de l'amidon et de

sa solution, tout comme les proportions d'amylase et d'amylopectine de plusieurs auteurs: entre autres, Maquenne et Roux (8), Gatin-Gruzewska (3), Ling et Nanji (5, 6), ou amylogène et amylon de Reich et Damansky (13).

Remerciement

Nous tenons à remercier M. l'abbé Alexandre Vachon, directeur de l'École Supérieure de Chimie, Université Laval, pour l'encouragement qu'il nous a accordé au cours de l'éxécution de ce travail.

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A STUDY OF THE VISCOSITY METHOD FOR THE DETERMINATION OF DAMAGE IN SILK[1]

By Audrey S. Tweedie[2]

Abstract

A method described by Trotman and Bell for the detection of damage in silk by measuring the viscosity of its solution in aqueous zinc chloride has been studied.

The effect of the temperature and the time of digestion on the viscosity has been investigated, and it is shown that a higher temperature with a shorter period of digestion gives equally satisfactory results, and is more convenient for a routine test.

The mechanism of the change in viscosity with time of digestion has been studied, and it is shown to be a dispersion process followed by hydrolysis of the silk.

Damage to weighted silk may be determined by this method if the silk is deweighted before dispersion in the zinc chloride solution.

The method has been used to determine the damage resulting from the treatment of silk with boiling dilute acid, boiling dilute alkali, light, and superheated steam. The viscosity of the damaged silk has been correlated with its tensile strength to give the viscosity method a quantitative basis. The amino nitrogen content of the silk was also determined and has been correlated with the tensile strength and viscosity. The results show that there is a difference between the hydrolysis of silk occurring in boiling dilute acid and that occurring during the corresponding alkali treatment. In the former, hydrolysis of the silk with formation of free amino groups occurs previous to dispersion, whereas in alkali it appears that the disintegration of the fibre into fibrils takes place very readily and that the fibrils are then rapidly dispersed in the alkaline solution before appreciable hydrolysis can occur. That the photochemical decomposition of silk is an oxidation process is confirmed. The action of steam appears to differ from that of acid, alkali, or light.

Introduction

Trotman and Bell (7) described a method for detecting damage in silk by measuring the viscosity of a zinc chloride solution of the silk. They applied the method to silk damaged by various chemical agents, *e.g.*, acid, alkali, and others, and later (8) reported results of an extension of the work, which included the determination of the effect of different degumming processes, dyeing, and the action of light.

Several interesting points in the papers mentioned (7, 8) appeared to merit further investigation. For example, the value of the method in the examination of faulty consumer goods would be greatly enhanced if it could be applied to weighted silks, which comprise most finished silk materials and garments. Furthermore, correlation of the change in viscosity of silk with change in other factors, such as its tensile strength under different conditions, *e.g.*, exposure to light, acid treatment, and other factors, would increase the usefulness of the viscosity test as a quantitative measure of damage. It was in order to investigate such points that the work described in this paper was undertaken.

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Materials and Methods

Silk Used

The following varieties of woven silk goods were used:

Silk *A*. A crêpe, containing no weighting or finishing materials. This piece had been stored in the dark since its purchase about two years prior to these experiments.

Silk *B*. A crêpe, containing weighting and finishing materials.

Silk *C*. A crêpe, containing finishing materials only.

Silk *D*. Parachute silk, plain weave, weight 2 oz. per sq. yd., containing no weighting or finishing materials.

Silk *E*. A crêpe, dyed blue, weighted, from an old silk cushion cover. This was so much rotted that it was falling apart.

Removal of Finishing and Weighting Materials from Silk

For the removal of finishing materials, the silk was extracted with petroleum ether, and then washed thoroughly by immersing in distilled water at a temperature of 60° C., removing, and squeezing. This was done a number of times and the process repeated in several changes of water.

For removal of weighting, the silk, after the finishing materials had been removed, was worked thoroughly in 2% hydrofluoric acid at 60° to 70° C. for 20 min. It was then rinsed in distilled water, worked well for 20 min. in a 2% sodium carbonate solution at 60° to 65° C. and finally thoroughly rinsed in distilled water.

Viscosity Measurement

The viscosity measurements were made with a 2.5% (weight per volume) solution of the silk in an aqueous zinc chloride solution of density 1.67 at 20° C. The weight of silk used was the conditioned weight at 65% relative humidity and 70° F. The weighed amount of silk, cut into small pieces, was placed in a small flask, and the required volume of zinc chloride solution added. The flask was then stoppered and placed in an oven at the given temperature for the required length of time with occasional swirling of the flask to ensure mixing of the contents*. It was then cooled in water to 20° C., the solution transferred to a viscosimeter (British Cotton Industries Research Association X-type viscosimeter), and the viscosity at 20° C. measured.

Tensile Strength Measurements

These were made with a Suter machine on material conditioned at 65% relative humidity and 70° F. The measurements were made in the warp direction only, and each value given in the table is the mean of 10 determinations carried out on strips 1 in. in width.

Amino Nitrogen Determination

The amino nitrogen content of the silk was measured by the Van Slyke method (9). A Van Slyke macro-apparatus was used with a 3 ml. gas burette substituted for the standard 40 ml. burette, as the volumes of nitrogen measured were small. For the reaction between nitrous acid and silk, 15 cc. of nitrous acid solution, 10 cc. of water (air free), and 5 cc. of silk solution

* *This operation is referred to in this paper as "digestion".*

were used instead of the 20 cc. of nitrous acid solution and the 10 cc. of protein solution specified in the original Van Slyke method. In place of the suspension of powdered silk used by other workers in the determination of amino nitrogen, the zinc chloride solution of silk (from viscosity determinations) was used. The amino nitrogen content of the silk is calculated as percentage of the dry weight of silk present in the solution.

Harris (1), in a study of the photochemical decomposition of silk, had determined the amino nitrogen content of silk after dissolving it in a 50% lithium bromide solution, but his later work was done on suspensions of finely powdered silk in distilled water. However, in their latest paper (5), Harris and co-workers mention the difficulty of preparing suspensions sufficiently uniform to assure the addition of an accurately known amount of protein for each determination. By using a zinc chloride solution of silk instead of an aqueous suspension, the weight of silk in a given volume is accurately known, but the actual volume delivered to the reaction chamber varies slightly with the viscosity of the solution, since the solution does not drain freely from the walls of the burette. However, the resulting error is small.

The zinc chloride solution causes the evolution of about three times as much nitric oxide as that caused by water, and this necessitated the use of an extra bulb below the gas pipette for storage of this gas before absorption. Difficulty with frothing and with the formation of a jelly-like mass of deaminized protein was experienced with the solutions of less-degraded silk.

Tests were made to determine the completeness of the reaction between the silk and the nitrous acid after various times of reaction, Table I. These showed that the reaction is virtually complete after five minutes; to provide a margin of safety a standard 6-min. shaking period was chosen.

The volume of nitrogen obtained in blank determinations was about half that obtained with undegraded or slightly degraded silk, *e.g.*, 0.41 and 0.79 cc. of nitrogen respectively. Thus, slight variations in the size of the blank resulted in relatively high variations in the calculated amino nitrogen content of the silk. Consequently, blank determinations were made for every sample of silk tested.

TABLE I

THE RATE OF REACTION BETWEEN NITROUS ACID AND SILK

Silk used	Time of reaction, min.	Amino nitrogen, % ± 0.02
Silk *D*—3½ hr. digestion at 45° C.	5	0.18
	10	0.20
	15	0.20
Silk *D*—18 hr. digestion at 45° C.	3	0.29
	5	0.31
	10	0.32
	14	0.29
Silk *D*—old solution–digested two to four months previously	3	1.20
	5	1.29
	10	1.35
	14	1.34

Kanagy and Harris (4) found variations in the quantity of nitrogen evolved with variation in the age of the sodium nitrite solution. In the present experiments it was found that much greater variations resulted from the alkaline permanganate solution used in the absorption pipette. This solution was made up from air-free water. To remove air inadvertently introduced, the solution was shaken in an evacuated flask before use. Even with these precautions the result of the first, and often of the second, blank, after the absorbing solution was changed, was high, and had to be discarded.

Results and Discussion

Effect of the Temperature and Time of Digestion on the Viscosity

Trotman and Bell (7) found that the viscosity of a solution of silk in zinc chloride depends on the temperature at which the silk is dissolved. In their early experiments they heated the silk with the solvent in a tube placed in boiling water, and then cooled the tube rapidly to 20° C., but they found it difficult to obtain a constant initial viscosity even when the heating period was timed with a stop-watch. They finally adopted the method of digesting the silk in the zinc chloride solution in a small stoppered bottle at 37° C. for exactly six hours, cooling to 20° C., and measuring the viscosity. The author of this paper found six hours a rather inconvenient length of time, as it was then necessary to make the viscosity measurements towards the end of the day; this prevented any further experimentation of a lengthy nature on those solutions that day. Trotman and Bell make no mention of trials at moderate temperatures other than 37° C., so it was decided to investigate the relation between the viscosity and the time and temperature of digestion, in the hope that a shorter period at a higher temperature might be equally satisfactory. Experiments were carried out at three temperatures, 37°, 45°, and 60° C., and with time intervals varying from 1 to 75 hr. The results are shown in Table II and Fig. 1.

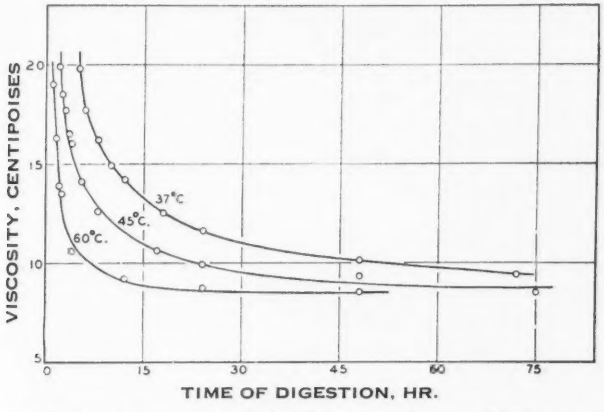

FIG. 1. *Variation of viscosity with time of digestion at three different temperatures.*

Some time after completion of this work, a translation was obtained of a paper by Tanizaki (6) on the regeneration of fibroin. In this paper, data are given for the relation between the time of digestion (10 to 120 min.) at 124° C. and the viscosity (in seconds) of the resulting zinc-chloride–fibroin solution. The curve obtained on plotting viscosity against time of digestion is similar to the curves obtained by the present author, although the concentration of fibroin used was somewhat lower.

TABLE II

EFFECT OF TEMPERATURE ON THE VARIATION OF VISCOSITY OF SILK *A* WITH TIME OF DIGESTION

Time of digestion, hr.	Temperature of digestion, °C.		
	37	45	60
	Viscosity, centipoises		
1	—	—	19.0
1.5	—	—	16.3
2	—	19.9	13.9
2.5	—	18.5	13.5
3	—	17.7	—
3.5	—	16.5	—
4	—	16.0	10.6
5	19.8	—	—
5.5	—	14.1	—
6	17.7	—	—
8	16.2	12.6	—
10	14.9	—	—
12	14.2	—	9.2
17	—	10.6	—
18	12.5	—	—
24	11.6	9.9	8.7
48	10.1	9.3	8.5
72	9.4	—	—
75	—	8.5	—

It will be seen that three hours' digestion at 45° C. gives the same viscosity as is obtained with six hours at 37° C. Moreover, the rate of decrease of viscosity with increasing time of digestion at 45° C. is only slightly greater than that at 37° C. at this part of the curve. At 60° C. the viscosity changes much more rapidly, and consequently very exact control of the digestion time would be necessary at this temperature. It was, therefore, decided to adopt the three-hour period of digestion at 45° C. for all further work.

From the figures given in Table II, it might be concluded that a two-hour period at 45° C. would be quite satisfactory. However, Silk *A* was somewhat degraded as a result of its two-year storage, and it dispersed fairly rapidly in the zinc chloride solution. With an undegraded silk such as Silk *D*, used in later experiments, a three-hour digestion period is necessary for the dispersion, and this period was therefore adopted as standard.

If from the curves shown in Fig. 1, the ratio of the time of digestion at 37° C. to that required at 45° C. to give the same viscosity is determined,

it is found that the ratio is virtually constant throughout the whole viscosity range, its average value being 2.2. The same holds true for the ratio of the time of digestion at 45° C. to that at 60° C., the average value in this case being 2.5. However, the experimental error at 60° C. is greater than that at the lower temperatures, and consequently the agreement between the various determinations of the ratio is not so good. The constancy of the two series of ratios is shown in Fig. 2, in which the three curves from Fig. 1 are replotted with the digestion periods at 37° C. and 60° C., calculated to their corresponding values at 45° C. For clearness, the logarithm of the time of digestion has been plotted against the viscosity. The three series of points fall closely on the same curve; this shows that the only effect of raising the digestion temperature is to increase the rate of reaction.

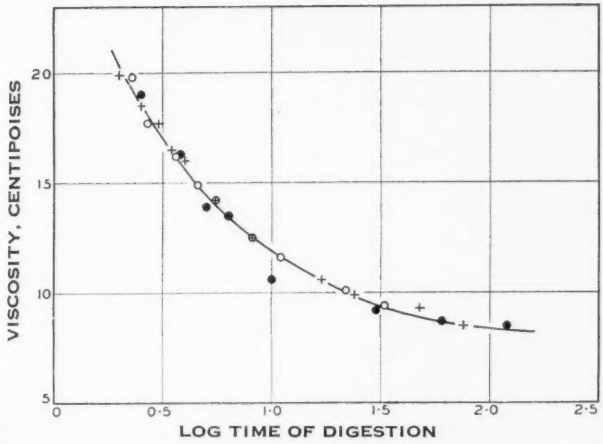

FIG. 2. +, *Digestion at 45° C.* ○, *Digestion at 37° C.; time of digestion ÷ 2.2 to give equivalent period at 45° C.* ●, *Digestion at 60° C.; time of digestion × 2.5 to give equivalent period at 45° C.*

An attempt was made to use a procedure for dissolving the silk similar to that adopted for the cuprammonium viscosity test on cotton. This involved placing the zinc chloride solution and the silk directly in the viscosimeter, together with two $\frac{3}{16}$ in. ball bearings to aid agitation, and then fastening the tube to a wheel that was made to rotate for 24 hr. at room temperature. The viscosity of a sample of Silk *A*, after this procedure, was 18.0 centipoises, which is very close to the figure of 17.7 as determined with the standard procedure. The quantity of silk used with the zinc chloride solution is several times that of the cotton used with the cuprammonium solution, and consequently occupies a large volume in the viscosimeter; thus it was difficult to mix the silk and the zinc chloride solution efficiently. Altogether this procedure is much less satisfactory than that involving digestion at a raised temperature.

Viscosity of Undamaged Degummed Silk

Trotman and Bell (7) concluded, from their results with Italian, Chinese, and Japanese silks, that the viscosity of a 2.5% solution of undamaged, degummed silk should not be less than 19.0 centipoises, and that a viscosity greater than 21.0 centipoises would indicate incomplete degumming. That the latter statement is not necessarily true is shown by the fact that the sample of Silk *D* examined by the present author had a viscosity of 23.7 centipoises. This silk as purchased was degummed, but a piece of the material was given a further treatment for one hour in a 25% (based on the weight of the silk) soap bath at 95° C. to obviate the possibility of the initial degumming being incomplete. The viscosity of the silk thus treated was 22.9 centipoises. The high viscosity of this silk may be explained by the high quality of the raw silk used and the careful processing involved in the manufacture of this type of material.

Application of the Method to Silk Containing Finishing and Weighting Materials

The only piece of silk on hand which contained finishing materials, but no weighting, was extracted with petrolic ether to remove any oily or waxy substances, and then dissolved in the zinc chloride. The resulting solution was very cloudy owing to the presence of suspended particles of those finishing materials not soluble in petrolic ether. These should, therefore, also be removed before the silk is tested by the viscosity method.

Weighted silk does not dissolve in zinc chloride solution and must, therefore, be deweighted before testing. It was found that the hydrofluoric acid deweighting treatment caused only a small drop in viscosity when applied to samples of unweighted silk. With weighted silk, the treatment would be expected to cause less damage, since the silk fibres are protected by the metallic salts during part of the time of treatment. The only sample of undamaged weighted silk (Silk *B*) that was on hand was deweighted by the hydrofluoric acid method, and its viscosity was then determined. The value of 22.0 centipoises that was obtained would certainly place this silk in the undamaged class.

TABLE III

VISCOSITY OF DEWEIGHTED SILKS AND THE EFFECT OF THE DEWEIGHTING TREATMENT ON THE VISCOSITY OF UNWEIGHTED SILK

Silk	Treatment	Viscosity, centipoises
C	Extracted and washed	19.8
C	Subjected to deweighting treatment	19.5
D	Untreated	23.7
D	Subjected to deweighting treatment	21.7
B	Deweighted	22.0
E	Deweighted	10.4
F	Deweighted	11.4
G	Deweighted	10.5

The method was also applied to the piece of rotted Silk *E*. After deweighting, the fabric was cut up finely and thoroughly mixed to ensure uniform sampling. As can be seen from Table III, the viscosity of the zinc chloride solution of this silk under the standard conditions was very low.

Two other samples of old, very rotten, weighted silk (Silks *F* and *G*) had viscosities very close to that of the blue silk already discussed.

Effect of Treatment with Sodium Chloride and Hydrochloric Acid

An attempt was made to damage silk with sodium chloride and hydrochloric acid solutions according to the procedure followed by Trotman and Bell (7) of soaking the silk in 10% sodium chloride for six hours or in 0.1 *N* hydrochloric acid for 12 hr., then squeezing and drying. The drying temperature was not specified, so this was taken to be room temperature. After drying, the silk was conditioned, thoroughly rinsed to remove free sodium chloride, dried, and reconditioned. Its viscosity was then determined. The results are given in Table IV.

TABLE IV

THE VISCOSITY OF SILK AFTER SODIUM CHLORIDE, HYDROCHLORIC ACID, AND HEAT TREATMENTS

Silk	Treatment	Viscosity, centipoises
*A**	Untreated	19.9
	Soaked 10 hr. in 10% NaCl, dried at 25° C.	19.3
	Soaked 12 hr. in 0.1 *N* HCl, dried at 25° C.	17.8
	Soaked 24 hr. in 0.1 *N* HCl, dried at 25° C.	16.5
	Soaked 3 hr. in 0.1 *N* HCl, dried at 105° C. for 3 hr.	12.5
	Soaked 0.5 hr. in 0.1 *N* HCl, dried at 105° C. for 1.5 hr.	13.3
	Soaked 0.5 hr. in 0.1 *N* HCl, dried at 105° C. for 24 hr.	12.6
	Kept at 105° C. for 72 hr.	17.5
	Boiled 18 min. in 0.1 *N* HCl, rinsed, dried at 25° C.	13.3
D	Original	23.7
	Wetted-out with distilled water, dried at 105° C. for 0.5 hr., repeated 3 times	22.1
	Boiled in distilled water for 20 min.	21.6

* *This work was done before the standard three-hour period of digestion was adopted. For this type of silk the two-hour period gave good results.*

The sodium chloride treatment caused little change in the viscosity of the silk, whereas Trotman and Bell noted a drop in viscosity from 21.8 to 18.3 centipoises under the same conditions.

The hydrochloric acid treatment caused a drop in viscosity from 19.9 to 17.8 centipoises, which is very small compared to the drop from 21.8 to 12.3 centipoises observed by Trotman and Bell. Increasing the period of soaking in the acid from 12 to 24 hr. and omitting the rinse, resulted in a further drop of 1.3 centipoises.

The viscosity of silk treated with hydrochloric acid and dried at 105° C. was in close approximation to that found by Trotman and Bell. Apparently then, heating is necessary in the treatment of silk with dilute hydrochloric acid to cause appreciable damage and the resultant large drop in viscosity. Heating untreated silk for 72 hr. at 105° C. caused a drop in viscosity of 2.4 centipoises, and it may, therefore, be concluded that for short periods at this temperature the change would be comparatively small. Three repetitions of the wetting in distilled water and drying for 30 min. at 105° C. each time also resulted in little change, as did boiling the silk in distilled water for

20 min. Varying the time of soaking in acid and the time of drying at 105° C. showed that most of the damage to the silk resulted during the short period of time necessary to actually dry the silk and evaporate the hydrochloric acid; *i.e.*, the hot hydrochloric acid caused the damage. This was verified by boiling the silk in 0.1 *N* hydrochloric acid for 18 min. The viscosity of the silk after this treatment was identical with that of the silk soaked in 0.1 *N* acid and dried at 105° C. for 1.5 hr.

Study of the Mechanism Resulting in Change of Viscosity with Increasing Time of Digestion

The relation between viscosity and time of digestion was studied for Silks *A*, *D*, and *E*. The results are given in Table V and are plotted in Fig. 3.

TABLE V

VARIATION OF VISCOSITY WITH TIME OF DIGESTION FOR SEVERAL SAMPLES OF SILK

Time of digestion, hr.	Viscosity at 20° C., centipoises		
	Silk *D*	Silk *A*	Silk *E*
1	—	—	10.8
2	—	19.9	10.7
2.5	—	18.5	—
3	23.7	17.7	10.4
3.5	20.8	16.5	—
4	—	16.0	10.3
5.5	—	14.1	—
6	15.3	—	—
7	—	—	10.1
8	—	12.6	—
10	—	—	9.6
12	12.4	—	—
15	—	—	9.2
17	—	10.6	—
18	—	—	—
24	10.1	9.9	9.0
48	9.2	9.3	8.4
72	9.2	—	8.4
75	—	8.5	—
97.5	8.6	—	—
240	8.3	—	—

It will be seen that with increasing age of, or damage to, the silk, there is a flattening of the curves toward the time axis; that is, the viscosity for any given period of digestion decreases with increasing degree of damage to the silk. The physical appearance of the zinc chloride solution of these silks shows a parallel change. After one and a half hours of digestion at 45° C., Silk *E* had completely dissolved and formed a clear solution, whereas the other silks still showed some fabric structure, and even after three hours of digestion their solutions had a frosty appearance due to the presence of minute particles of silk fibre which were visible under the microscope. Furthermore, Silk *D*, which had the highest viscosity under the standard conditions, required the longest digestion period to give a clear solution.

It appears from these observations that the change in viscosity over the first portion, at least, of the curve obtained by plotting viscosity against time of digestion represents a change in the degree of dispersion of the silk in the zinc chloride solution. A natural assumption would be that the silk fibre is gradually broken down into its ultimate units through rupture (by the mechanical forces involved in swelling) of the bonds holding these units together in the fibre structure. Theoretically, the viscosity at this

stage would be the true viscosity of a solution of silk "molecules" in zinc chloride solution. At this period, and probably earlier, there is the possibility of the silk molecule being attacked chemically by the products of hydrolysis of a salt such as zinc chloride. Acid hydrolysis resulting in the formation

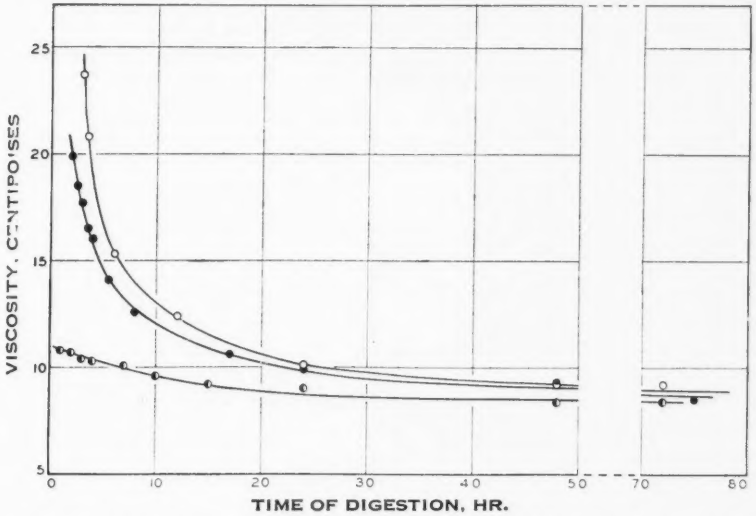

FIG. 3. *Relation between viscosity and time of digestion at 45° C. for Silks A, D, and E, of different age. Top curve, Silk D; middle curve, Silk A; bottom curve, Silk E.*

of free amino groups could be expected. It was decided to check this possibility by determining the amino nitrogen content of the zinc chloride solution of Silk *D* after different times of digestion, and to compare these figures with those from samples of Silk *D* which had been hydrolyzed to varying degrees by boiling with dilute hydrochloric acid and then subjected to the standard viscosity treatment. The relation between viscosity and amino nitrogen content of these two series of silk solutions is shown in Tables VI and VII and in Fig. 4.

TABLE VI

THE VISCOSITY AND AMINO NITROGEN CONTENT OF SILK *D* AFTER VARIOUS PERIODS OF DIGESTION

Heating period		Viscosity, centipoises	Amino nitrogen, %
Hr.	Temp., °C.		
3	45	23.7	0.22
3.5	45	20.8	0.20
6	45	15.3	0.20
12	45	12.4	0.28
18	45	10.6	0.31
24	45	10.1	0.48
48	45	9.2	0.62
97.5	45	8.6	0.95
240	45	8.3	1.82
72	37	9.6	0.53
168	37	8.8	0.75

It will be seen that both series of figures lie on the same curve. Unfortunately

the highest amino nitrogen content observed for the silk boiled in hydrochloric acid was only 0.48%. When the silk was given more drastic treatment with the acid it fell to pieces. There is no increase in amino nitrogen until the viscosity of the silk solution drops below 15 centipoises. Beyond this point, decrease in viscosity is accompanied by increase in amino nitrogen content until the viscosity reaches a limiting value, while the amino nitrogen content continues to increase. Two solutions of silk digested at 37° C. gave results that fall on the same curve. It will be noted that there is no

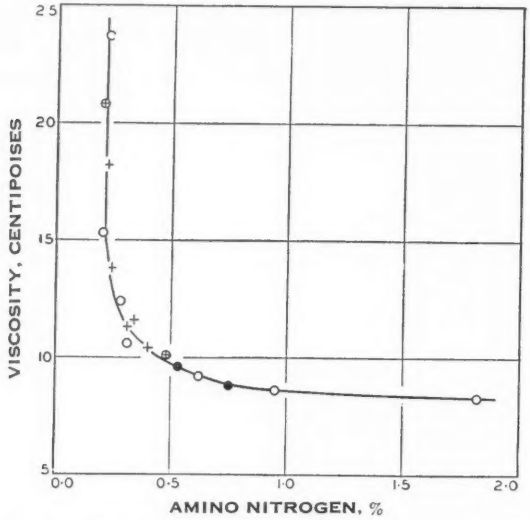

FIG. 4. *Relation between viscosity and amino nitrogen content of Silk D.* ○, *after various periods of digestion at 45° C.* ●, *After various periods of digestion at 37° C.* +, *Acid treated silk.*

TABLE VII

THE TENSILE STRENGTH, VISCOSITY, AND AMINO NITROGEN CONTENT OF SILK *D* AFTER TREATMENT WITH DILUTE HYDROCHLORIC ACID

Acid treatment		Viscosity, centipoises	Amino nitrogen, %	Tensile strength, lb. per in.
Time of boil, min.	HCl conc., normality			
0	0.0	23.7	0.22	47.0
5	0.05	20.9	0.20	45.5
5	0.1	18.2	0.22	44.5
20	0.1	13.8	0.24	41.5
5	0.5	11.6	0.34	39.5
6	0.6	11.3	0.31	34.5
7	0.7	10.4	0.40	23.5
6	0.8	10.5	—	19.5
5	0.9	10.2	0.48	9.0
5	1.0	9.9	0.49	7.5

change in amino nitrogen content when the digestion period is less than six hours; this indicates that there is no appreciable degradation of the silk in the three hour digestion period adopted as standard.

The experimental results recorded here appear to justify the hypothesis advanced in the earlier part of this section. Trotman and Bell (7) noted the fall in viscosity on prolonged digestion at 37° C. and on keeping the solution at room temperature, but stated that it did not appear to be due to hydrolysis. They dialyzed a zinc chloride solution of silk (age not given) and found neither peptones nor amino acids in the dialysate. However, the dilution involved in dialysis might easily mask the presence of such small amounts of amino nitrogen as are present.

Relation Between Viscosity and Tensile Strength of Silk Damaged in Various Ways

This relation was investigated for samples of Silk *D* which had been damaged (*a*) by boiling with hydrochloric acid of various concentrations, (*b*) by exposure in a fugitometer at an average temperature of 36° C., (*c*) by exposure in a fugitometer at an average temperature of 96° C., (*d*) by boiling with sodium hydroxide of various concentrations, and (*e*) by steaming at a pressure of 15 lb. for various periods. The results are given in Tables VII, VIII, IX, X, and in Fig. 5.

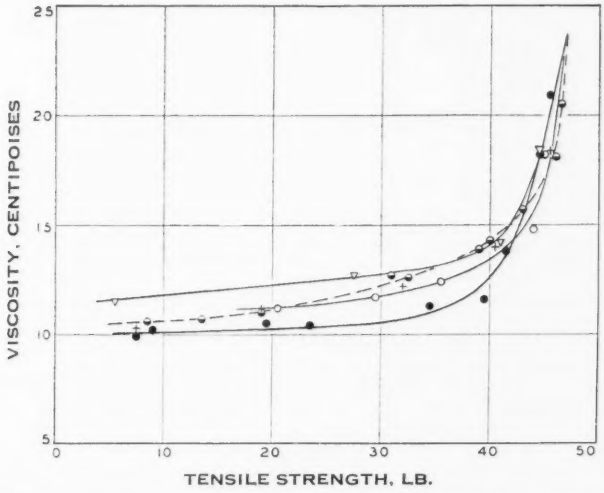

FIG. 5. *Viscosity–tensile-strength relation for Silk D damaged by:— steaming under pressure, ○; dilute sodium hydroxide, ▽; dilute hydrochloric acid, ●; exposure in fugitometer at 36° C. (◒) and 96° C. (+).*

Although the results obtained with each of the treatments give slightly different curves, all four curves are of the same general type, and the viscosity method can, therefore, be used to detect damage of a chemical or photochemi-

TABLE VIII

THE TENSILE STRENGTH, VISCOSITY, AND AMINO NITROGEN CONTENT OF SILK EXPOSED IN A FUGITOMETER AT TWO DIFFERENT TEMPERATURES FOR VARIOUS PERIODS

Silk	Time of exposure, hr.	Viscosity, centipoises	Tensile strength, lb. per in.	Amino nitrogen, %
D, exposed at 36° C.	0	23.7	47.0	0.20
	3	20.5	46.5	—
	6	18.1	46.0	—
	12	15.7	43.0	—
	18	14.3	40.0	—
	24	13.9	39.0	—
	36	12.6	32.5	—
	48	12.7	31.0	—
	72	11.0	19.0	—
	97	10.7	13.5	0.18
	120	10.6	8.5	0.19
D, exposed at 96° C.	2	18.4	45.5	—
	8	14.0	40.5	—
	17	12.2	32.0	—
	24	11.2	19.0	—
	48	10.3	7.5	—

TABLE IX

THE TENSILE STRENGTH, VISCOSITY, AND AMINO NITROGEN CONTENT OF SILK *D* TREATED WITH DILUTE ALKALI

Alkali treatment		Viscosity, centipoises	Tensile strength, lb. per in.	Amino nitrogen, %
Time of boil, min.	NaOH conc., normality			
5	0.05	18.4	44.5	—
5	0.1	14.2	41.0	—
5	0.15	12.7	27.5	—
5	0.25	11.5	5.5	0.21

TABLE X

THE TENSILE STRENGTH, VISCOSITY, AND AMINO NITROGEN CONTENT OF SILK *D* STEAMED AT 15 LB. PRESSURE

Time of steaming, hr.	Viscosity, centipoises	Tensile strength, lb. per in.	Amino nitrogen, %
3	18.2	45.0	—
8	14.8	44.0	—
18	12.4	35.5	—
26	11.7	29.5	0.21
40	11.2	20.5	0.22

cal nature to silk. The initial large drop in viscosity is accompanied by only a small change in tensile strength; these results parallel those obtained with cotton with the cuprammonium viscosity method. This makes the viscosity method especially valuable, in that damage is readily detected even when the tensile strength shows little change, a condition that would likely obtain when the processing methods are faulty. The method may also be used to differentiate between chemical damage and that caused by mechanical means.

Differences in the Action of Certain Agents that Cause Damage to Silk

(i) Dilute Acid

As has already been mentioned, boiling hydrochloric acid causes hydrolysis of silk, with a resulting increase in its amino nitrogen content. This is accompanied by a decrease in tensile strength and a slight yellowing of the silk. The relation between the amino nitrogen content of the acid-damaged silk and its viscosity has been shown in Fig. 4. In Fig. 6 the tensile strength of the silk is plotted against its amino nitrogen content. It will be seen that down to the point where the silk has lost half of its original strength, the amino nitrogen content may be considered as varying linearly with the tensile strength. With further hydrolysis the change in amino nitrogen content is less rapid than that in tensile strength.

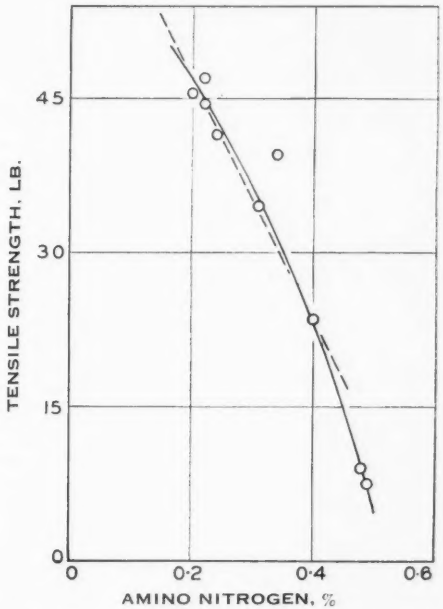

FIG. 6. *Relation between tensile strength and amino nitrogen content of silk damaged by dilute hydrochloric acid.*

(ii) Light

Harris (1) showed that the amino nitrogen content was not changed during the deterioration of silk by the action of light in the presence of oxygen. This fact has been confirmed by the writer with samples of silk exposed in the fugitometer for 97 and 120 hr., respectively. The results are given in Table VIII.

By reference to Fig. 5, it can be seen that the relation between viscosity and tensile strength of silk exposed in the fugitometer at 36° C. is the same as that of silk exposed at 96° C. In other words, the action taking place under the two sets of conditions is similar. In Fig. 7 the tensile strength of the silk is plotted against the time of exposure in the fugitometer for both temperatures; a much more rapid decrease in tensile strength at the higher temperature is shown. The form of the curves is very similar to that of the curves obtained by Harris and Jessup (2) with untreated silk exposed at 70° C.

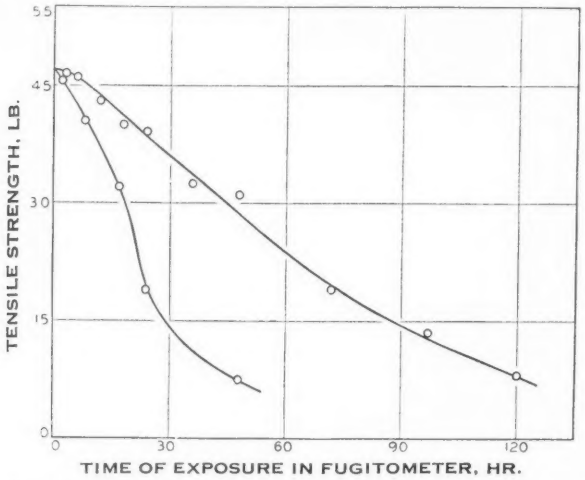

FIG. 7. *Relation between tensile strength and time of exposure of Silk D in a fugitometer. Upper curve, 36° C.; lower curve, 96° C.*

In Fig. 8 the logarithm of the time of exposure is plotted against the logarithm of the viscosity of the silk for both temperatures. A straight line relation is obtained, and, moreover, the two lines are parallel, except for a short distance at their upper ends. At 36° C. $\log V/\log T$ increases during the initial period of exposure until it reaches a certain value that remains constant during further exposure, whereas at 96° C. $\log V/\log T$ initially decreases until it attains its constant value. At both temperatures, approximately the same time (1.5 to 2 hr.) is required for attainment of this constant value.

The relative humidity of the air in the fugitometer was not controlled in these experiments, and consequently the moisture content of the silk would be quite different at the two temperatures used. The action of the two factors, temperature and humidity, cannot therefore be differentiated in this experiment.

Exposure to light in the fugitometer causes the white silk to become yellowish. While the viscosity measurements on these silks were being made, it was noted that there was a definite gradation in the color of the zinc chloride

solutions with time of exposure. The color varied from water-white through yellow to light yellow-brown, the color deepening with increasing time of exposure to light. This color may be due directly to the modified silk or to small amounts of some secondary product which may be split off under the action of light.

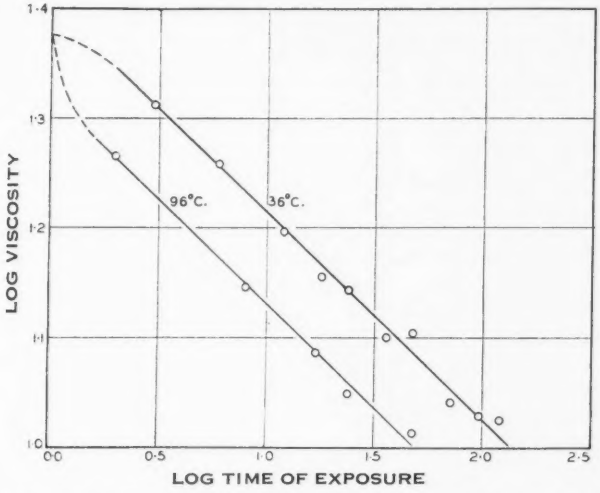

FIG. 8. *Relation between logarithm of viscosity and logarithm of the time of exposure of Silk D in a fugitometer at two different temperatures.*

(iii) Dilute Alkali

The action of a boiling dilute solution of sodium hydroxide on silk is quite different from that of boiling dilute hydrochloric acid. When silk is boiled in dilute sodium hydroxide there is a loss in weight, which increases with increasing concentration of the alkali. The loss is very marked when the silk is boiled for five minutes in 0.25 *N* sodium hydroxide, whereas the weight of silk is little changed when it is boiled for the same length of time in hydrochloric acid solution of even four times this normality. The decrease in tensile strength of silk fabric boiled in dilute alkali is due partly therefore to the loss of silk, whereas with the acid treatment, the loss in tensile strength appears to be almost wholly due to modification of the molecular structure of the silk fibre. This is indicated by its increasing amino nitrogen content. The amino nitrogen content of the sample of silk that had been boiled in the 0.25 *N* sodium hydroxide solution was unchanged, although its viscosity and tensile strength were low.

It has been stated by Jordan Lloyd and Marriott (3) that the swelling of silk fibroin in dilute alkaline solutions is accompanied by a splitting of the fibre into fibrils and by a softening of the protein. From the present experiments, it appears that in the boiling alkali this disintegration into fibrils

takes place very rapidly in the more exposed fibres, and that these fibrils are then speedily dispersed in the alkaline solution before the alkali can cause their hydrolysis to any appreciable extent. In other words, alkaline hydrolysis of the silk "molecules", with resulting formation of free amino groups, takes place only after the silk has been dispersed, whereas in the acid hydrolysis, the formation of free amino groups occurs previous to dispersion. Jordan Lloyd and Marriott (3) have previously suggested that the splitting of the fibre into fibrils in acid solution probably takes place at a plane of cleavage different from that in alkaline solution.

(iv) Steaming

Silk may be hydrolyzed also by steaming under pressure for prolonged periods. It appeared interesting to ascertain whether the mechanism of the early stages of damage to silk resulting from such steaming shows any resemblance to that involved in the early stages of acid or alkali damage. Samples of Silk *D* which had been steamed under 15 lb. pressure (250° F.) for 26 and 40 hr., respectively, were tested for their amino nitrogen content. The results are given in Table IX. The change in amino nitrogen content is so small as to be of doubtful significance. The only conclusion that can be drawn from this very limited experimentation is that the breakdown of fibre structure by steaming under pressure is, in the early stages at least, different from that caused by boiling with dilute acid. Judged from the appearance of the silk, the damage is also different from that caused by alkali, notably in the freedom of the silk from the stiffness, translucency, and thinning that resulted from alkali treatment, and in the pronounced brown coloration that develops on steaming. In the last named respect, it more closely resembles silk that has been exposed for several days in the fugitometer.

Altogether, the results given in this section fit in very well with the view advanced by Jordan Lloyd and Marriott (3), that different reagents may be expected to attack different "faults" in the crystal structure of a protein fibre, such as silk, and to influence the properties in different ways.

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THE STRUCTURE OF DEXTRAN SYNTHESIZED BY *LEUCONOSTOC DEXTRANICUS*

Investigation of dextran (*L.d.*) obtained by the action of *Leuconostoc dextranicus* on sucrose, and carefully purified by electrodialysis, followed by methylation and hydrolysis, has led to the surprising result that the hydrolysis products are free from tetramethyl methyl glucoside, within the limits of experimental accuracy. The principal product of hydrolysis is 2,3,4-trimethyl methyl glucoside, but in addition about 10% of a dimethyl methyl glucoside is also formed.

The identity of the dimethyl methyl glucoside has not yet been completely established, but inasmuch as the synthesis of the three dimethyl methyl glucosides in question is approaching completion, this problem should be clarified in the near future.

The significance of these results lies in the fact that no evidence of the presence of any "end groups" has been found; this proves that we are not dealing with a short chain polymer either with or without side chains. On the contrary the accumulated evidence points to the substance being a linear polymer in which the terminal units of one chain are in chemical union with members of adjacent chains to form a network-like structure. In such a case the sugar representing the terminal groups would be present in such small quantity in the hydrolytic products as to render its detection almost impossible.

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March 17, 1938.

THE ISOLATION OF GUAIACOL AND PYROGALLOL 1,3-DIMETHYL ETHER FROM HARDWOOD WASTE SULPHITE LIQUOR

Treatment of a hardwood waste sulphite liquor (obtained by pulping a mixture of beech, birch, and maple woods), with sodium hydroxide (9%) at 150° C., yields, in addition to acetosyringone (1), guaiacol and pyrogallol 1,3-dimethyl ether.

Guaiacol was identified as its *p*-nitrobenzoyl ester (melting point, 104° to 105° C.; mixed melting point, 104° to 105° C.). Pyrogallol 1,3-dimethyl ether was identified by comparison of the *p*-nitrobenzoyl ester (melting point, 155° to 156° C.; mixed melting point, 155° to 156° C.) and cörulignon oxidation products with those obtained from an authentic sample of the material.

Investigation of a corresponding volatile phenol fraction from birch lignin sulphonic acid is in progress.

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